

GABA and Sleep

Molecular, Functional and Clinical Aspects

Jaime M. Monti
S. R. Pandi-Perumal
Hanns Möhler
(Editors)

 Springer

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ISBN 978-3-0346-0225-9

e-ISBN 978-3-0346-0226-6

DOI 10.1007/978-3-0346-0226-6

Library of Congress Control Number: 2010935596

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Cover Illustration: Distribution of $\alpha 1$ GABA_A receptors shown immunohistochemically in mouse brain. Courtesy of Jean-Marc Fritschy, Institute of Pharmacology, University of Zurich

Cover design: deblik, Berlin

Printed on acid-free paper

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Dedicated to our wives and families

Foreword

Insomnia has plagued mankind since time immemorial. For a long time, plant remedies were the only pharmacological means of obtaining some relief. The situation changed with the discovery of synthetic hypnotics. The barbiturates were introduced at the beginning of the twentieth century and remained the most popular compounds for 50 years. Their era came to a close with the discovery of the sleep-promoting properties of the benzodiazepines. Currently, we look back on another 50-year period dominated by this class of hypnotics. Benzodiazepines and their analogs have still not relinquished their status as the most widely used sleep-promoting agents. It is interesting that the hypnotic properties of both the barbiturates and the benzodiazepines were discovered by chance and that their mechanism of action remained obscure for a long time. Today we know that they act via the promotion of GABA, the principal inhibitory neurotransmitter in the brain. While the functions of GABA became increasingly clear after the discovery of its role in the central nervous system in the early 1950s, its implication in sleep regulation remained unknown. A major milestone was the identification of the binding site of benzodiazepines by Möhler and Okada in 1977. It soon became obvious that benzodiazepines exert their action by promoting the synaptic release of GABA indirectly by binding to an allosteric site of the receptor complex. Thanks to the recent work of the Möhler team; we know today that their hypnotic action is mediated principally by the $\alpha 1$ GABA_A receptor, which is one of the several receptor subtypes.

Does the unabated popularity of the benzodiazepine receptor agonists imply that they come close to the ideal sleeping pill? This is not the case, since their untoward actions limit their use. These include abuse, tolerance, rebound insomnia, memory impairment, ataxia, and daytime impairment of performance. Also the sleep EEG does not show the typical features of physiological sleep. There is no doubt that the introduction of benzodiazepines and their analogs represented major progress in the treatment of insomnia, since the agents used previously had a less propitious profile. However, the advances in the last decades were mainly due to the development of agents with more favorable

pharmacokinetic properties and to their increasingly judicious and circumspect application in therapy.

The ideal hypnotic would be a compound promoting physiological sleep. Is there an endogenous sleep promoting substance? The search for such an agent was initiated at the beginning of the last century by Henri Piéron in France and Kuniomi Ishimori in Japan. They reported sleep-inducing effects after infusing cerebrospinal fluid or brain extracts from sleep-deprived dogs into naïve animals. In the meantime, a large number of endogenous compounds with sleep-promoting properties has been identified. They include hormones, prostaglandins, cytokines, and purine nucleosides. However, none of these agents were shown to play a central role in physiological sleep regulation. Moreover, a translation from the experimental level to the clinical level has not occurred.

The approach focusing on neurotransmitters and neuromediators appears to be more promising. Antagonists at receptors of the serotonin, histamine, and orexin system and agonists at receptors of the melatonin and GABA_A system were shown in clinical trials to have hypnotic properties. Moreover, some of these drugs affected the EEG in ways resembling physiological sleep intensification. However, despite these interesting developments, the predominant position of benzodiazepine receptor agonists is unlikely to be soon challenged.

In addition to their hypnotic properties, benzodiazepines also have anxiolytic, anticonvulsant, and central muscle relaxant effects. Moreover, some agents are used as intravenously administered short-acting anesthetics. This wide range of actions may hamper their therapeutic use as hypnotics. One way to enhance the selectivity is to develop agents targeting specific subtypes of the GABA_A receptor. This strategy may lead to advances by allowing an ever more subtle modulation of the GABA system. In view of such developments, it is most welcome that the present volume is devoted to the molecular, functional, and clinical aspects of GABA and sleep. The editors, renowned experts in their field, have compiled an impressive series of authoritative overviews from experienced basic and clinical scientists. By providing a bridge between basic physiology and pharmacology, sleep science, and therapeutics, the volume will be useful to experts and students from a wide variety of disciplines.

University of Zurich

Alexander Borbély

Preface

The increase in our knowledge of the GABA (γ -aminobutyric acid) system in recent years has led to major advances in our understanding of sleep physiology, sleep disorders and clinical sleep medicine. The goal of this first edition is to review the major achievements made in characterizing the role of GABA in the physiology and pathology of sleep regulation and in identifying GABAergic subsystems which show potential for novel pharmacological treatments of sleep disorders.

Brain states such as sleep or waking are governed by distinct synchronized oscillatory neuronal networks which give rise to changing EEG patterns. It has increasingly been recognized that the sculpting of neuronal rhythms, the control of neuronal firing and the selection of temporary assemblies of neurons are controlled by a rich diversity of GABAergic interneurons. In addition, mutual inhibition between the brain nuclei which promote sleep and the arousal systems is known to result in switching properties that define waking and sleep states. In this process, which is driven by homeostatic and circadian influences, GABA neurons play also a significant role. The evidence reviewed in this volume clearly demonstrates that GABAergic regulatory control is at the center of sleep physiology, pathophysiology and therapeutics.

To accommodate the diverse temporal dynamics of GABAergic signaling, a corresponding diversity of GABA receptors is required on the target cells. Besides the metabotropic GABA_B receptor, the fast-responding ionotropic GABA_A receptors are of special relevance in as much as they represent the exclusive target of benzodiazepine (BZD) drugs. More recently, the discovery of GABA_A receptor subtypes, largely characterized by distinct subunits, has opened up new opportunities for drug development. For instance, sedation, a common denominator of GABA_A receptor-related hypnotics, is mediated by $\alpha 1$ receptors, while $\alpha 2$ receptors selectively mediate the anxiolytic action of BZD.

Additionally, our understanding of the pharmacokinetic determinants (absorption rate, elimination half-life, dosage) of hypnotic drug action has progressed in parallel with the development and clinical use of a series of BDZ and non-BDZ hypnotic drugs.

The BDZ derivatives reduce sleep latency, the number of nocturnal awakenings, and wake time after sleep onset. Increases in total sleep time are related to greater amounts of stage 2 (intermediate sleep). By contrast, stage 3 and 4 sleep (deep sleep) and REM sleep (dream sleep) are decreased in patients with chronic primary insomnia and comorbid insomnia. On the other hand, the non-BZD derivatives zolpidem, zopiclone, eszopiclone and indiplon, which primarily act selectively at $\alpha 1$ GABA_A receptors, increase total sleep time without reducing slow wave sleep and REM sleep. Interestingly, the selective extrasynaptic GABA-receptor agonist gaboxadol increases slow wave sleep, that is, it mimics the effect of sleep deprivation.

The intricate nature of sleep regulation via the GABA system is particularly apparent in nuclei of the arousal system such as the dorsal raphe nucleus (DRN), the locus coeruleus and the pontine cholinergic system. In the DRN, local administration of the GABA_A receptor agonist muscimol increases REM sleep, whereas the local microinjection of serotonin 5-HT_{1B}, 5-HT_{2A/C}, and 5-HT₇ receptor agonists induces the opposite effect. This result has been ascribed to the inhibition of GABAergic interneurons and the activation of long-projection GABAergic cells, respectively. GABA also plays a critical neuromodulatory role in the interaction between pontine noradrenergic and cholinergic systems. Cholinergic mechanisms are important for REM sleep induction in the pontis oralis and sublaterodorsal nuclei, and GABAergic modulation of these sites can inhibit or prevent the occurrence of REM sleep.

Organization of the First Edition

This volume is divided into three major parts. Part I: Basic Physiology and Pharmacology; Part II: Sleep Science and Circuitry; and finally, Part III. Hypnotics.

The volume consists of 20 chapters and covers a broad range of topics related to the basic, pharmacological, and clinical aspects of GABA and sleep.

Part I consists of an overview of the basic pharmacology of the GABAergic system. The topics covered include the most recent understanding concerning the pharmacology and physiology of the GABAergic system and its receptor subtypes, the development of subtype-selective GABA_A receptor compounds for the treatment of insomnia, anxiety and epilepsy, distribution of GABA_A receptor subtypes in the human brain, and finally, the pharmacokinetic determinants of the clinical effects of benzodiazepine agonists.

Part II is the largest section in this volume and addresses the topic of sleep circuitries. These include sleep and its modulation by drugs that affect the GABA_A receptor function, subcortical neuromodulation of feedforward and feedback inhibitory microcircuits by the reticular activating system, and circadian regulation of sleep. This section also addresses the role of melatonin in sleep and the possible involvement of GABAergic mechanisms.

Part III addresses the pathophysiology of sleep disorders, differential diagnosis of insomnia, and safety and efficacy profiles of various GABAergic drugs, including zolpidem, zopiclone, eszopiclone, zaleplon, and Indiplon.

This volume is intended for pharmacologists, CNS drug discovery scientists, basic and clinical researchers, psychiatrists, and other general practitioners who

seek an overall grasp of the physiology and clinical pharmacology of sleep. It will be helpful for medical students and graduate students of biomedical and sleep medicine specialties.

The information presented is based on the most recent sleep literature and stresses the relevance to clinical medicine and therapeutics. Information about specific drugs is occasionally repeated in several chapters throughout this volume by various authors. It is the editors' hope that this redundancy will be construed as a benefit.

We welcome communication from our readers concerning this volume and its organization, and especially concerning any inaccuracies or omissions that remain. We take full responsibility for any such inaccuracies, and we appreciate having them drawn to our attention.

In summary, it is our hope that this volume will enable interested scientific and medical researchers to develop a better understanding of the science and practice of sleep medicine. We also hope that this volume will generate new ideas that lead to improvements in the care of patients who suffer from sleep disorders.

Uruguay
Canada
Switzerland
September 2010

Jaime M. Monti
S.R. Pandi-Perumal
Hanns Möhler

About the Editors

Jaime M. Monti MD has won many awards for his research, including the Claude Bernard Award (Clinical Sleep Research) from the Government of France and the Schering Award for Basic Sleep Research in Germany. He is a member of the International College of Neuropsychopharmacology, Sleep Research Society (USA), European Sleep Research Society, and the Argentinian Society of Sleep Medicine.

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Credits and Acknowledgements

This volume owes its final shape and form to the assistance and hard work of many talented people. Creating a book, which surveys a broadly interdisciplinary field such as sleep medicine, involves the collaborative scholarship of many individuals. We express our profound gratitude to the many people who have helped in this endeavor.

Our sincere appreciation goes to Professor Borbely, who graciously agreed to write the foreword. The editors also wish to express their sincere appreciation and owe endless gratitude to all our distinguished contributors for their scholarly contribution that facilitated the development of this volume. Our largest debt obviously goes to our outstanding authors who, regardless of how busy they were, managed to find time for this project. They, in a most diligent and thoughtful way, have brought a wide range of interests and disciplines to this volume.

It is of course a pleasure to thank our many colleagues who commented on individual chapters and have provided invaluable suggestions: we are indebted to them all.

We would like to thank the secretarial and administrative staffs of our respective institutions, for helping us to stay on task and for their attention to detail.

We acknowledge with gratitude the work of the editorial department of Birkhauser-Verlag. We are especially indebted to Dr. Beatrice Menz, Senior Commissioning Editor – Medicine, who was an enthusiastic and instrumental supporter from the start. We also thank the Birkhauser-Verlag production department colleagues for their meticulous work.

A very special debt of gratitude and appreciation is owed to the several reviewers who made numerous helpful suggestions during the conception of this project.

Last, but certainly not the least, we are most grateful to our wives and families, who provided invaluable support.

To all the people who contributed to this volume, we express our sincere gratitude.

Uruguay
Canada
Switzerland

Jaime M. Monti
S.R. Pandi-Perumal
Hanns Möhler

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Part I

Basic Physiology and Pharmacology

Physiology and Pharmacology of the GABA System: Focus on GABA Receptors

Hanns Möhler

Abstract Wake and sleep states have long been known to be implemented by distinct synchronized neural oscillations. Changes in the pattern of neural oscillations have recently been recognized to be largely due to the impact of GABAergic regulation. Inhibitory interneurons are the main players in sculpting neuronal rhythms, controlling spike timing, selecting network assemblies and implementing brain states. A rich diversity of GABAergic interneurons imprints its activity, mediated through a comparably rich diversity of GABA_A receptors. Pharmacologically, there is a clear division of labor among GABA_A receptor subtypes. Sedation, a common denominator of GABA_A receptor-related hypnotics, is mediated via α_1 GABA_A receptors. However, the hypnotic EEG finger print of diazepam is largely linked to α_2 GABA_A receptors, pointing to two distinct receptor systems for sleep regulation. Anxiety is a major impairment of sleep, which can be selectively controlled by α_2 GABA_A receptor modulators. Chronic pain, another frequent sleep impediment can be alleviated by α_2 /GABA_A receptor modulators. Chronic pain, another frequent sleep impediment can be alleviated by α_2/α_3 GABA_A receptor modulators. Finally, cognitive deficits can be pharmacologically addressed by partial inverse agonists of α_5 GABA_A receptors. Thus, in the future, it is conceivable that disease-specific hypnotics could be developed by combining the modulation of suitable GABA_A receptor subtypes. GABA_B receptors play a pharmacological role as target of γ -hydroxybutyrate, which is frequently used in the treatment of narcolepsy. Thus, as our understanding of GABAergic deficits in sleep disturbances increases, the strategic targeting of GABA receptor subtypes may represent a new approach for the personalized pharmacological management of sleep disorders.

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1 Neural Oscillations and GABA

Over the past several decades, GABAergic inhibition has been recognized to play a central role in the brain circuitries that regulate the daily cycles of sleep and wakefulness [1]. Mutual inhibition between the arousal and sleep-promoting circuitry results in switching properties that define wake and sleep states. This process is under homeostatic influence (“need to sleep”) and circadian drive [2]. The present review focuses on the physiology and pharmacology of GABA receptors and their ligands in the promotion of sleep.

Sleep promotion through the ventrolateral preoptic area (VLPO) of the anterior hypothalamus is effectuated by neurons that produce GABA and the inhibitory neuropeptide galanin. They project to the aminergic ascending arousal system of the brain stem (coeruleus and raphe) and the histaminergic wake-promoting tuberomammillary nucleus (TMN) of the hypothalamus. VLPO neurons also receive afferents from each of the major monoaminergic systems. Thus, the VLPO can be inhibited by the very arousal systems that it inhibits during sleep. These circuits contain mutually inhibitory elements and contribute to the “flip-flop switch” between wake and sleep under the influence of yet unidentified homeostatic factors (somnogens) [1, 3, 4].

Different brain states are associated with distinct synchronized oscillatory neural activities, which give rise to distinct EEG patterns. Alpha waves are prominent when the eyes are closed and subjects are in a relaxed state (“cortical idling”). Sleep and the transition between different stages of sleep are characterized by different EEG patterns [4], which reflect the ability of cortical neurons to oscillate synchronously in various frequency bands such as β , δ , or theta-waves or spindles. The computational advantage of synchronized neural oscillations is that it orchestrates the spike firing of neurons in discrete time windows. The assumption is that an activity that is synchronized with millisecond precision summates more effectively than a nonsynchronized activity and thereby favors processing of the selected responses or brain states.

In the distributive architecture of the brain, functionally related areas require a mechanism to communicate. Synchronized oscillations are thought to be the major mechanism to coordinate interactions of large numbers of neurons that are distributed within or across different specialized brain areas. The best studied example of a transient synchronization of neural discharges is the gamma frequency band (30–80 Hz), which is thought to be a neuronal correlate of a cognitive content (e.g., in sensory perception) or an executive program (e.g., motor response), in which the synchronization is presumed to link the neural networks involved.

Complex brains have developed special mechanisms for the grouping of principal cells into temporal coalitions of local or distant networks. It largely consists of the inhibitory neuron “clocking” network [5] i.e. GABAergic interneurons, which temporally regulate pyramidal cell activity (Fig. 1). In cortical circuits, the inhibitory neurons control spike timing of principal cells, sculpt neuronal rhythms, select cell assemblies, and implement brain states. To achieve this regulatory control of the relatively uniform pyramidal cells, GABA interneurons display a rich diversity,

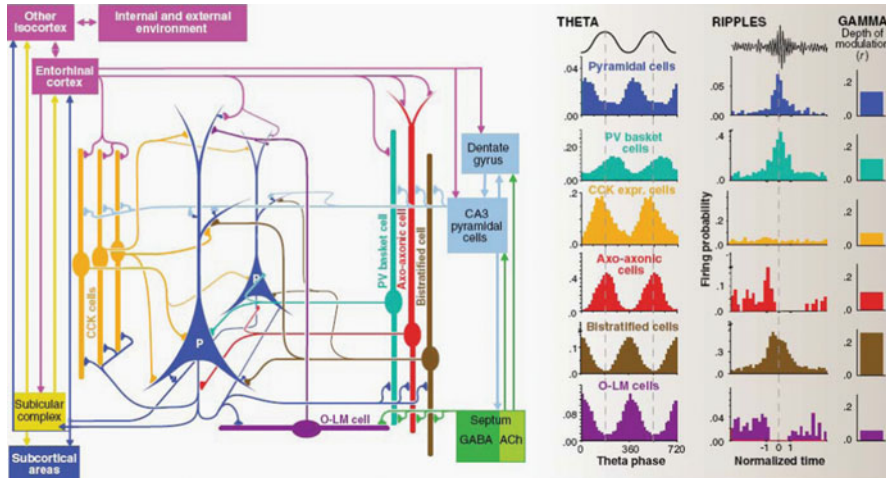


Fig. 1 Spatiotemporal interaction between pyramidal cells and several classes of interneurons during network oscillations, shown as a schematic summary of the main synaptic connections of pyramidal (P, blue), PV-expressing basket (green), axo-axonic (red), bistratified (brown), O-LM (violet), and three classes of CCK-expressing interneurons (yellow). The firing probability histograms (right part) show that interneurons innervating different domains of pyramidal cells fire with distinct temporal patterns during theta and ripple oscillations, and their spike timing is coupled to field gamma oscillations to differing degrees. The same somatic and dendritic domains receive differentially timed input from several types of GABAergic interneuron Ach, acetylcholine. The figure is taken from [6]

which imprints a GABAergic conductance matrix on pyramidal cells. The interneurons innervate discrete subcellular domains of pyramidal cells such as the soma, the axon initial segment, or dendrites, and act in discrete time windows to achieve the computational sophistication [6]. Since cortical interneurons receive not only local input but are also under modulatory control from subcortical areas such as brain stem nuclei and hypothalamus, the wake-sleep regulation areas can impinge on the oscillatory patterns by modulating GABAergic control.

2 Role of GABA Receptors

The dynamics of GABAergic control in different frequencies require GABA receptors, which are able to faithfully transmit the temporal dynamics of transmitter signaling. GABA receptors are differentially expressed to suit the particular neural circuit and are structurally diverse to accommodate the temporal demands. There are two major types of GABA receptors, ionotropic GABA_A receptors and metabotropic GABA_B receptors. GABA_A receptors are pentameric ligand-gated chloride ion channels, which mediate the major inhibitory responses and act prominently in oscillation control (7–9). GABA_B receptors are dimeric G-protein-coupled

receptors, which largely affect excitatory and inhibitory neurotransmitter release. Both receptors, but GABA_A receptors in particular, have been recognized to be crucial for an understanding of the physiology and pathology of major brain systems and for the development of drugs for a host of neurological and psychiatric diseases [7–9]. The present review focuses on the role of the GABA receptors in the regulation of sleep and in the pharmacotherapy of sleep disorders.

3 GABA_A Receptors and their Multiplicity

Based on the presence of seven subunit families comprising at least 18 subunits in the CNS (α_{1-6} , β_{1-3} , γ_{1-3} , δ , ϵ , θ , ρ_{1-3}), the GABA_A-receptors display an extraordinary structural heterogeneity (Fig. 2). Nevertheless, all subunits exhibit a similar topology with a large extracellular N-terminal domain (~200 amino acids), four α -helical transmembrane segments (M1–M4), a large intracellular loop connecting transmembrane segments 3 and 4, and a short extracellularly located C-terminal

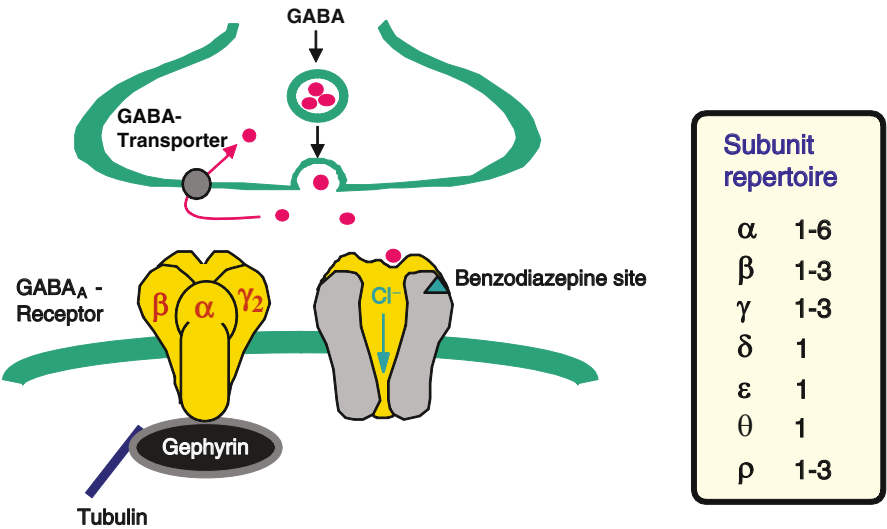


Fig. 2 Scheme of GABAergic synapse depicting major elements of signal transduction. The GABA_A receptors represent GABA-gated chloride channels and are heteromeric membrane proteins, which are linked (directly or indirectly) to the synaptic anchoring protein gephyrin and the cytoskeleton (Tubulin). The sequence of subunits corresponds to a modeling proposal [10]. The binding sites for GABA and benzodiazepines are located at the interface of α/β and α/γ_2 subunits, respectively. Synaptic GABA_A receptors mediate phasic inhibition providing a rapid point-to-point communication for synaptic integration and control of rhythmic network activities. Extrasynaptic GABA_A receptors (not shown) are activated from synaptic spillover or nonvesicular release of GABA. They mediate tonic inhibition and provide a maintenance level of control of neuronal excitability [11, 12]

sequence. Within the extracellular N-terminal domain, all subunits contain a 15 amino acid long disulfide-linked loop, which is characteristic for all members of the Cys-loop superfamily, which also includes the glycine-, the nicotinic acetylcholine-, and the 5HT₃-receptor as oligomeric ligand-gated ion channels.

With few exceptions, GABA_A receptors are heteropentamers composed of isoforms of three types of subunits, α , β , and γ (Fig. 2) [13–15]. The structural diversity of GABA_A receptors means that they have a range of differences in their channel kinetics, affinity for GABA, rate of desensitization, ability for transient chemical modification such as phosphorylation, cell-type-specific expression, and – in the case of multiple receptors in a neuron – a domain-specific location. This confers to each receptor considerable adaptive flexibility to meet the requirements of the interneuron-associated circuits [11].

4 Benzodiazepine-Sensitive GABA_A Receptors

Receptors containing the α_1 , α_2 , α_3 , or α_5 subunits in combination with the β_2 or β_3 subunit and the γ_2 subunit are most prevalent in the brain. These receptors are sensitive to modulation by classical benzodiazepines, such as diazepam, which continue to represent a major class of hypnotics and anxiolytics. The most prevalent GABA_A receptor subtype is composed of $\alpha_1\beta_2\gamma_2$ subunits with only a few brain regions lacking this receptor (e.g., granule cell layer of the olfactory bulb, reticular nucleus of the thalamus, spinal cord motoneurons) [8].

Receptors containing the α_2 or α_3 subunit are considerably less abundant and are highly expressed in brain areas where the α_1 subunit is absent or present at low levels. The α_2 receptors are particularly prominent on the axon initial segment of pyramidal cells in the cortex and hippocampus and also represent the major GABA_A receptor in the central nucleus of the amygdala. The α_3 GABA_A receptors are the main subtypes expressed in monoaminergic and basal forebrain cholinergic cells [43], and are, in addition, located in the thalamic reticular nucleus, and thus strategically positioned for modulating thalamic oscillations [44]. Receptors containing the α_5 subunit are less widely distributed in the brain but are expressed to a significant extent extrasynaptically on pyramidal cells of the hippocampus, where they are predominately coassembled with the β_3 and γ_2 subunits. Receptors containing the δ -subunit are exclusively extrasynaptic, supporting tonic inhibition. The molecular factors regulating GABA_A receptor assembly, domain-specific insertion, and recycling are increasingly being recognized [45].

It should be kept in mind that complex benzodiazepine actions, such as the development of tolerance, can implicate more than a single receptor subtype. For instance, while the sedative action of diazepam is mediated by α_1 GABA_A receptors (see below), the development of tolerance to this action under chronic diazepam treatment requires the interaction with both α_1 GABA_A receptors and α_5 GABA_A receptors [46]. In general, classical benzodiazepine drugs, such as diazepam don't distinguish GABA_A receptor subtypes by affinity of intrinsic activity [40].

Table 1 GABA_A receptor subtype ligands

Drug	Main activity	Interaction with recombinant GABA _A receptors ^{a,b}	References
A. Benzodiazepine site ligands			
Zolpidem	Hypnotic	Preferential affinity for α_1	[16]
Zaleplone	Hypnotic	Preferential affinity for α_1	[16]
Indiplon	Hypnotic	Preferential affinity for α_1	[17]
L-838 417	Anxiolytic	Comparable affinity at α_1 , α_2 , α_3 , α_5 subtype. Partial agonist at α_2 , α_3 , α_5 (not α_1) subtype	[18]
	Antinociceptive		[19]
Ocinaplon	Anxiolytic	Comparable affinity at α_1 α_2 , α_3 , α_5 subtype. Partial agonist at α_2 , α_3 , α_5 subtype, nearly full agonist at α_1	[20]
SL 651 498	Anxiolytic	Agonist at α_2 , α_3 , partial agonist at α_1 and α_5 subtype	[21]
	Antinociceptive		[22]
TPA 023 (MK0777)	Anxiolytic	Partial agonist at α_2 , α_3 subtypes, antagonist at α_1 , α_5 subtypes	[23]
TPA 003	Anxiolytic	Partial agonist at α_3 subtype, acts at high receptor occupancy	[24]
ELB 139	Anxiolytic	Selective receptor profile uncertain	[25]
NS 11394	Anxiolytic	Comparable affinity at α_1 , α_2 , α_3 , α_5 Partial agonist at α_2 , α_3 subtype, nearly full agonist at α_5 subtype	[26]
	Antinociceptive		[27]
α_3 1A	Anxiogenic	Weak inverse agonist at α_3	[28]
L-655 708	Memory enhancer,	Partial inverse agonist with preference for α_5 subtype	[29, 30]
α_5 1A	Memory enhancer	Partial inverse agonist with preference for α_5 subtype	[31–33]
Ro 493851	Memory enhancer	Partial inverse agonist with preferential affinity and efficacy at α_5 receptors	[34]
B. Modulatory sites other than benzodiazepine site			
Ethanol	Anxiolytic	High sensitivity (≥ 3 mM) at $\alpha_4(\alpha_6)\beta_3\delta^c$; Medium sensitivity (≥ 30 mM) at $\alpha_4(\alpha_6)\beta_2\delta^c$; Low sensitivity (≥ 100 mM) at $\alpha_4(\alpha_6)\beta_3\gamma_2$	[35]
Neurosteroids (e.g. 3 α ,5 α -THDOC)	Sedative Anxiolytic Sedative Anesthetic	High sensitivity at δ -containing subtypes ^c and at α_1 , α_3 receptors in combination with β_1	[36]

Intravenous anesthetics (Etomidate Propofol)	Sedative Anesthetic	Act on receptor subtypes containing β_3 i.e. mainly on α_2 and α_3 subtypes	[37]
C. GABA site ligand			
Gaboxadol	Hypnotic Antinociceptive	Partial agonist at α_1 , α_3 subtypes, full agonist at α_5 and superagonist at $\alpha_4\beta_3\delta$ receptors ^c	[38] [39]
^a Classical partial agonists, which do not differentiate between GABA _A receptor subtypes such as Bretazenil [40] or Pagoclone [41], are not considered in this review			
^b Data should be treated with caution as properties of recombinant receptors that are expressed in foreign host cells might not give an accurate reflection of their neuronal counterparts			
^c GABA is a weak partial agonist on δ -containing receptors, which largely explains the strong modulatory response of ligands acting on δ -containing receptors [42]. THDOC, 5 α -pregnane3 α ,21-diol-20-one			

5 The Benzodiazepine Site, a Servo Mechanism for Inhibition

Due to their strictly allosteric GABA_A receptor interaction, classical benzodiazepines, such as diazepam display special properties [92]. The drugs promote an exclusively ancillary function by enhancing the affinity for GABA. As a consequence, more receptors can be recruited for activation by GABA, which results in an enhanced response to a given concentration of GABA. The dose response curve for GABA is shifted to the left. However, in some synapses, the amplitude of the GABA response (mIPSC) remains unaltered by a benzodiazepine agonist, which indicates that all GABA_A receptors are saturated by a single quantum of GABA. Thus, the maximal response to GABA cannot be exceeded in the presence of the drug; when all available receptors are activated by GABA, there is no further drug-induced enhancement possible. It is this feature that provides the clinical safety of benzodiazepines. In addition to increasing the GABA-induced postsynaptic current, benzodiazepines also prolong the decay of the postsynaptic current (mIPSC). This effect likewise contributes to the enhancement of the GABA response by a benzodiazepine drug. The mechanisms are valid not only for the classical benzodiazepines, e.g., diazepam, but also for the newer “non-benzodiazepine” ligands acting at the benzodiazepine site (e.g., zolpidem, eszopiclone) with a preferential affinity for α_1 receptors.

6 Diazepam-Insensitive GABA_A Receptors

GABA_A receptors that do not respond to clinically used ligands such as diazepam, flunitrazepam, clonazepam, or zolpidem are sparsely distributed in the brain and are largely characterized by the α_4 and α_6 subunits in combination with a β subunit and the γ_2 or δ subunit. Receptors containing the δ -subunit are generally extrasynaptic (see below).

7 GABA_A Receptors and Sleep Promotion

Neural oscillations are the “middle ground” between the activity of single neurons and behavior. Wake and sleep stages are characterized by EEG patterns of different frequencies [1, 3, 4]. Also, sleep homeostasis, the tendency to maintain sleep propensity, has a correlation in the EEG (EEG power in the theta frequency range [5–8 Hz]) [47]. However, the link between neuronal oscillations and their molecular control with regard to sleep is largely unclear. With the increasing recognition that oscillatory networks are under GABAergic control (see above), the role of GABA_A receptor subtypes in inducing and maintaining sleep has become a major focus of studies aiming to develop novel and more refined hypnotics.

Benzodiazepines and chemically distinct congeners such as zolpidem or zaleplon remain among the most widely used drugs in the treatment of sleep

disturbances. The term “sedative-hypnotic” with regard to benzodiazepine drugs is frequently taken to imply that sedative and hypnotic action is mediated by the same receptors or circuits. This view is not supported when the molecular basis of benzodiazepine-induced sedation and the EEG profile is compared. The sedative component of benzodiazepines, measured by the reduction of locomotor activity, is attributed to neuronal circuits expressing α_1 GABA_A receptors, the most prevalent receptor subtype in the brain amounting to ~60% of all GABA_A receptors. Experimentally, mice in which the α_1 GABA_A receptor had been rendered diazepam-insensitive by a point mutation [α_1 (H101R)] failed to be sedated by diazepam or zolpidem [18, 48, 49]. The prominent role of α_1 GABA_A receptors is particularly evident in the control of the thalamic gateway of information transfer to the cerebral cortex. The early stages of sleep are associated with a powerful reduction of cortical oscillatory activity from the 40 Hz awake state to light sleep (7–15 Hz). Thalamo-cortical relay cells, which relay information to the cortex, are inhibited through GABAergic input from thalamic reticular neurons. These relay cells express α_1 GABA_A receptors [44]. Similarly, histaminergic cells of the arousal system contain α_1 GABA_A receptors. In keeping with this view, not only classical benzodiazepines but also ligands with preferential affinity for α_1 receptors, such as zolpidem, zaleplon, or indiplon, act as sedative-hypnotics (Table 1). Conspicuously, gaboxadol (THIP), an agonist acting at the GABA site of δ -receptors, has failed in clinical studies to reduce sleep latency [for review 50].

8 GABA_A Receptor Subtypes and EEG Patterns

While benzodiazepine-induced sedation was attributed to α_1 GABA_A receptors, it was surprising that the EEG pattern induced by diazepam, such as the change in sleep architecture (suppression of REM sleep), EEG-frequency profiles (reduction of slow-wave sleep, increase in fast β -frequencies), and in sleep contiguity (decrease of the number of brief awakenings), was found to be largely due to effects mediated by receptors other than α_1 since these properties remained largely unaffected in α_1 (H101R) mice [51]. Thus, the profile of the sleep-promoting effects of diazepam is mediated largely by receptors other than α_1 . It appears that the most pronounced effect of diazepam on sleep, as measured by EEG recordings in wild-type mice, is derived from the enhancement of α_2 GABA_A receptors. When the α_2 GABA_A receptors were rendered diazepam-insensitive by a point mutation [α_2 (H101R)], the diazepam-induced suppression of δ -waves (0.75–4 Hz) in NREM sleep, the increase in fast β -waves in non-REM sleep (>16 Hz), and the diazepam-induced increase of theta-waves (6–11 Hz) in REM sleep were strongly attenuated [52]. Thus, the hypnotic EEG profile of diazepam and its sedative action seem to be controlled by different neuronal circuits.

Remarkably, the effects of zolpidem on the sleep EEG in mice are distinct from changes typically induced by diazepam. In wild type mice, non-REM sleep EEG power was markedly and dose-dependently reduced by zolpidem in a broad

frequency band (>5 and 9 Hz after 5 and 10 mg zolpidem, respectively). In the α_1 (H101R) mutants, the non-REM sleep power reduction was absent or limited (depending on the dose), suggesting that zolpidem-induced sleep promotion, including its EEG profile, is primarily mediated via α_1 GABA_A receptors [53]. This result is in keeping with the preferential affinity of zolpidem for α_1 GABA_A receptors (intermediate affinity for α_2 - and α_3 receptors and lack of affinity for α_5 receptors). Thus, while diazepam and zolpidem share their interaction with α_1 receptors (mediating sedation and thereby reducing time to sleep onset), they differ strongly in their GABA_A receptor-related EEG profile. The consequences of this distinction for sleep quality remain to be evaluated.

9 Role of α_3 GABA_A Receptor Subtypes in the Arousal Systems

The neurons in the ascending arousal systems express, at least in part, α_3 GABA_A receptors as shown immunohistochemically for the locus coeruleus and the nucleus raphe and basal forebrain cholinergic neurons [54, 55]. Sleep promotion was therefore expected to be achieved via α_3 GABA_A receptors, induced by the GABAergic output of the hypothalamic VLPO to the arousal-promoting locus coeruleus and raphe, and possibly also the tuberomammillary nucleus (TMN) of the hypothalamus and the orexin neurons. The wake-promoting histamine neurons in the TMN were found to express α_2 receptor mRNA; unfortunately, the α_3 subunit mRNA was not measured in the respective study. In the thalamus, α_3 GABA_A receptors are prominent in the reticular nucleus, which controls the inhibition of the thalamic relay cells and thereby controls cortical afferents. Indeed clonazepam was found to suppress thalamic oscillations through α_3 but not α_1 GABA_A receptors [44]. α_3 GABA_A receptors would therefore appear to be a promising target for hypnotic drug action. However, in point-mutated mice with diazepam-insensitive α_3 -containing GABA_A receptors, the EEG profile of diazepam was not different from that seen in wild-type animals [56], implying that neurons expressing α_3 GABA_A receptors do not substantially contribute to the EEG pattern. In animals that lacked the α_3 subunit gene, only a slight elevation of spontaneous locomotion was found [57]. Furthermore, TPA003, a benzodiazepine site agonist, which acts selectively on α_3 GABA_A receptors (Table 1), did not display sedative activity [24] even at high receptor occupancy. Thus, the role of α_3 GABA_A receptors in sleep regulation is not obvious at present despite its prominent localization in sleep-related brain areas.

10 Role of δ GABA_A Receptors and Gaboxadol (THIP)

Classical benzodiazepines attenuate low frequency EEG activity and suppress REM sleep. These characteristics are not in line with physiological sleep and hypnotics which resemble physiological sleep intensification are being sought.

GABA_A-receptor subtypes were therefore investigated with the aim of enhancing physiological sleep and thereby improving sleep quality. The GABA-mimetic gaboxadol (4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridine-3-ol hydrochloride; synonym THIP), which interacts preferentially with recombinant $\alpha_4\beta_3\delta$ GABA_A receptors at the GABA site [38, 58] (not at the benzodiazepine site!), was found to enhance delta or slow-wave (0.75–4 Hz) activity, hallmarks of physiological NREM sleep. This was interpreted to indicate a drug-induced increase in sleep pressure as shown in humans, rats, and rabbits [50, 59]. In situ, δ -subunit-containing receptors are located exclusively extrasynaptically (see above). The EEG effects of gaboxadol were absent in mice that lacked the δ -subunit-containing receptor [60]. Thus, a promising new hypnotic drug target seemed to have been discovered. However, in wild type mice, gaboxadol induced an abnormal EEG pattern as shown by a dramatic increase in spectral power in the low-frequency range. Since this effect was similar in waking and in NREM sleep, the drug did apparently not specifically affect sleep regulation [61]. The clinical development of gaboxadol by Lundbeck/Merck was recently discontinued for undisclosed reasons. A failure of the compound to reduce sleep latency, an activity attributed to α_1 GABA_A receptors, may have been a contributing factor (see Steiger, this volume).

11 Anxiolytic Activity Without Sedation

Anxiety is an essential emotional response, which, when exacerbated, can result in anxiety disorders for which drugs acting at the benzodiazepine site are frequently used therapeutically. Anxiety is an impediment to sleep. A hypnotic agent that incorporates anxiolytic activity would therefore be desirable. The differentiation of GABA_A receptors by knock-in point mutations showed that it was the α_2 - but not the α_1 -, α_3 -, or α_5 GABA_A receptor that mediated the anxiolytic-like activity of diazepam [62, 63]. With the α_2 GABA_A receptor, a highly selective target for the anxiolytic activity of benzodiazepine tranquilizers had been identified. In keeping with this notion, the benzodiazepine site ligand L-838417, which has shown efficacy at α_2 , α_3 , and α_5 but not α_1 GABA_A receptors, showed anxiolytic activity in wild type rats [18] (Table 1). Similarly, other partial agonists with efficacy at α_2 , α_3 , and/or α_5 receptors, but not at α_1 receptors, were found to show anxiolytic activity in rodents [26, 64] (Table 1). The contribution of α_3 GABA_A receptor to the anxiolytic activity is less obvious. An α_3 -selective inverse agonist was anxiogenic and proconvulsant in rodents (Table 1) [64]. However, in mice that lacked α_3 GABA_A receptors, the anxiolytic activity of diazepam was undiminished [57]. Nevertheless, TP003, a ligand with selective efficacy at α_3 GABA_A receptors, was anxiolytic, albeit only at a receptor occupancy, which was much higher than that required for the anxiolytic activity of classical benzodiazepines [24]. Thus, the α_2 GABA_A receptors appears to be the major mediator of anxiolytic activity [8, 12, 65], although the α_3 receptor may contribute at high receptor occupancy. The α_2 receptors take up strategic positions mainly on the soma and the axon initial

segment of pyramidal cells, e.g., in cerebral cortex and hippocampus, and also occur in the amygdala. This domain-selective inhibitory control seems to be a key requirement for promoting anxiolytic activity [8, 66, 67]. Among ligands of the α_2/α_3 receptors, TPA 023 was tested clinically not only in patients with anxiety disorders (see Attack, this volume) but also with schizophrenia (see below). The future development of hypnotic agents may incorporate ligands with activity at $\alpha_2\text{GABA}_A$ receptors, i.e., reducing anxiety, a currently underutilized strategy for promoting sleep.

12 Pain Suppression via Spinal α_2/α_3 GABA_A Receptors

Chronic pain is a frequent impediment to sleep. Spinal α_2 (and α_3) GABA_A receptors were recently identified as powerful gate keepers against pain [19]. The experimental α_2/α_3 GABA_A receptor ligand L-838 417 (Table 1) was highly effective in suppressing inflammatory and neuropathic pain, yet devoid of unwanted sedation and motor impairment. Most importantly, in contrast to morphine, L-838417 failed to show tolerance in analgesic efficacy, as tested over a treatment period of 9 days [19]. The α_2/α_3 receptor ligand reduced not only the nociceptive input but also the activity in brain areas associated with the associative-emotional component of pain, as demonstrated by fMRI in rodents [19]. Similarly, NS11394, which acts as partial agonist at α_2 and α_3 receptors and as almost full agonist at α_5 receptors, was effective in rat models of inflammatory and neuropathic pain [27] (Table 1). These results provide a rationale for the development of a new class of antinociceptive and antiinflammatory drugs, which utilize pain-related inhibitory interneuron networks in the brain and spinal cord [68]. Such a strategy, when combined with α_1 -receptor activity, would provide promising personalized hypnotics for patients suffering from chronic pain.

13 Targeting Cognitive Impairment Through $\alpha_5\text{GABA}_A$ Receptors

Spatial and temporal memory is linked to oscillatory neuronal activity in the hippocampus. The glutamatergic input on pyramidal cells is balanced by GABAergic synaptic and tonic inhibition. Surprisingly, tonic inhibition via $\alpha_5\text{GABA}_A$ receptors was found to regulate learning and memory. When α_5 receptors were partially reduced genetically, associative learning in a hippocampus-dependent memory task was enhanced [63]. Similarly, spatial memory in water maze learning was enhanced in mice lacking α_5 receptors completely [69]. Thus, for the first time, a defined GABAergic control of temporal and spatial memory had been established. Indeed, partial inverse agonists acting at $\alpha_5\text{GABA}_A$ receptors have been found to enhance the cognitive and executive performance of wild-type rodents and monkeys [29, 34]

(Table 1) and have reduced the ethanol-induced memory-deficit in healthy volunteers [31]. The clinical utility of α_5 partial inverse agonists for the treatment of cognitive deficits remains to be determined.

14 Cognitive Enhancement Through α_2 and α_3 GABA_A Receptors

A putative role for α_2/α_3 receptor ligands in reversing a cognitive deficit became apparent in schizophrenia patients. Among the various clinical symptoms of schizophrenia [70], the cognitive deficits are associated with a deficit in EEG gamma band activity [93, 94], pointing to a potential deficit in GABAergic control. Indeed, in post mortem studies, GABAergic Chandelier neurons in frontal brain were found to be dysfunctional [71]. These neurons are known to act via α_2 GABA_A receptors [72]. Furthermore, in mice, a deficit of α_3 GABA_A receptors resulted in sensorimotor gating deficits and a hyperdopaminergic phenotype [57]. These results pointed to a new GABAergic strategy for treating deficits in cognition and sensorimotor gating in schizophrenia. Enhancing the GABAergic drive was hypothesized to restore oscillatory synchronicity and cognitive function in schizophrenia. MK0777 (TPA 023), a ligand of the benzodiazepine site with efficacy at α_2/α_3 GABA_A receptors (Table 1), was tested in a proof of concept study [73]. A 4 week treatment with MK 0777, as an add-on to the standard antipsychotic therapy, resulted in an increased level of attention and working memory and showed a trend in restoring the oscillatory power of the gamma band, when the patients performed a cognitive task. Thus, a GABA substitution therapy might be effective in reconstituting the phenotype and the biotype of cognition. This example is expected to trigger further clinical trials to potentially support an entirely new therapeutic concept, based on α_2/α_3 GABA_A receptors for the treatment of cognitive deficits [73]. Investigations on the impact of MKO777 treatment on sleep in these patients would be desirable.

15 Dependence Liability of GABA_A Receptor Drugs

Compared to classical benzodiazepine drugs which don't differentiate among receptor subtypes, drugs acting at GABA_A receptor subtypes were expected to show a lower risk of dependence liability. Comparative studies have been performed in nonhuman primates using a drug discrimination and a self-administration procedure [74–76]. In these studies, squirrel monkeys or baboons with implanted catheters were trained to discriminate triazolam from saline based on a food reinforcement schedule. Subsequent testing with a particular test drug was continued up to doses that engendered 80% or more of responses on the drug-appropriate lever (full substitution). The α_1 GABA_A receptor preferring drugs

zolpidem or zaleplon, like the nonselective diazepam, fully substituted for triazolam [74, 75]. In contrast, L-838 417, which displays efficacy as partial agonist at α_2 , α_3 , and α_5 GABA_A receptors but acts as antagonist at α_1 receptors, did not substitute for triazolam [75]. Similarly, in a self-administration procedure, in which rhesus monkeys had been trained to self-administer the short acting barbiturate methohexital under a progressive ratio schedule, zolpidem permitted the maintenance of near maximum performance. In contrast, L-838 417 maintained self-administration just significantly above vehicle levels [75]. These results suggest that stimulation of α_1 GABA_A receptors is essential for promoting the abuse potential of these drugs [90, 95].

This view is supported by an analysis of other subtype selective compounds. SL 651 498 displays anxiolytic-like effects and acts as full agonist at α_2 and α_3 GABA_A receptors while displaying only partial agonistic efficacy at α_1 - and α_5 GABA_A receptors (Table 1). Correspondingly, SL 651 498 only partially substituted for triazolam in squirrel monkeys trained to discriminate triazolam from saline [77]. Furthermore, the compound TPA 023, which displays partial agonistic activity at α_2 and α_3 receptors but lacks agonistic activity at α_1 and α_5 receptors, was devoid of dependence liability when tested either in the drug discrimination paradigm or the self-administration paradigm [23, 76]. This result is more encouraging as TPA 023, which has been tested clinically (see above), displays robust anxiolytic activity in the squirrel monkey conditioned emotional response assay, and is devoid of sedation. In rodents, TPA 023 lacked precipitated withdrawal symptoms after chronic treatment (following administration of FG 7142), but displayed anxiolytic-like and anticonvulsant activity [23]. Thus, for ligands acting as partial agonists at α_2 and α_3 GABA_A receptors, a pronounced pharmacological efficacy is compatible with a much reduced or absence of dependence liability and withdrawal symptoms. These findings clearly demonstrate that the development of α_1 -sparing ligands is the most promising strategy to minimize or alleviate the dependence liability and the withdrawal potential for benzodiazepine site ligands [76]. The discovery of this strategy is a major step forward in reducing or alleviating dependence liability of benzodiazepine site ligands.

16 GABA_B Receptors

16.1 Structure and Physiology of GABA_B Receptors

In contrast to the rich structural diversity of the ionotropic GABA_A receptors, GABA_B receptors are G-protein-coupled receptors that exist as heterodimers of two subunits, GABA_{B1} and GABA_{B2} [78]. GABA_{B1} contains the GABA site, GABA_{B2} provides the mechanism for coupling to G proteins. GABA_B receptors occur in only two subtypes, based on two GABA_{B1} variants, the subunit GABA_{B1a} and GABA_{B1b}, both of which combine with the GABA_{B2} subunit to form two types

of heteromeric GABA_{B(1a,2)} and GABA_{B(1b,2)} receptors. Most neurons coexpress GABA_{B1a} and GABA_{B1b}. They differ in amino acid sequence only by a pair of short repeats at the N-terminus which are present in GABA_{B1a} but not in GABA_{B1b} [78].

GABA_B receptors located on GABAergic nerve terminal are referred to as autoreceptors, and, on all other presynaptic terminals, GABA_B receptors are referred to as heteroreceptors. Depending on whether GABA_B receptors are activated on excitatory or inhibitory terminals, their effects on the postsynaptic neuron are inhibitory or disinhibitory, respectively. The physiological response to GABA_B receptor activation is mediated via Ca²⁺-channels or K⁺-channels [78]. Inhibition of neurotransmitter release via GABA_B receptors is effectuated by inhibiting Ca²⁺ channels. Postsynaptic GABA_B receptors induce a slow inhibitory postsynaptic potential (IPSP_B) by activating Kir-3 type K⁺ channels with the consequence of hyperpolarizing the membrane and shunting excitatory currents. Thus, postsynaptic GABA_B receptors can restrict long-term potentiation (LTP), whereas activation of GABA_B autoreceptors promotes the induction of LTP. In dendrites, GABA_B receptor activation inhibits dendritic Ca²⁺ channels and thereby prevents local spike activity [79] and influences synaptic plasticity. Finally, GABA_B receptors are known to inhibit adenylate cyclase (AC) via the G α i/o subunits of G-proteins. Unfortunately, the physiological consequences of inhibiting AC are still poorly understood. In general, as recent studies have demonstrated, most GABA_B receptors are located far from the GABA release sites and probably require an accumulation of synaptically released GABA to be activated. For instance, inhibitors of GABA uptake are required to enhance the IPSP_B through GABA_B receptor activation in the control of rhythmic hippocampal activity [80].

16.2 GABA_B Receptors and Orexin Neurons

The most direct evidence for a role of GABA_B receptors in sleep/wake control is the regulation of the hypothalamic orexin (hypocretin)-producing neurons. Orexin neurons receive innervations from several brain regions including the limbic system, preoptic area and monoaminergic neurons. They also receive glutamatergic input and GABAergic innervation from local interneurons. Electrophysiologically, orexin neurons have been shown to be inhibited via both GABA_A- and GABA_B receptors in brain slices. In mice, in which GABA_B receptors were lacking selectively in orexin neurons, the sensitivity of orexin neurons to both excitatory and inhibitory inputs was decreased due to an augmented GABA_A inhibitory tone, which shunted postsynaptic currents in these neurons [81]. Behaviorally, these mice exhibited a severe fragmentation of sleep/wake states during both the light and the dark periods, without showing an abnormality in total sleep time or cataplexy. Thus, GABA_B receptors on orexin neurons appear to be crucial for stabilizing the state-switching mechanisms.

16.3 GABA_B Receptors as Target for Gamma-Hydroxybutyrate

Cataplexy and excessive daytime sleepiness, associated with narcolepsy (hypocretin deficiency), are frequently treated with gamma hydroxybutyrate (GHB; sodium oxybate). Nightly doses of GHB have been shown to reduce the number of nocturnal awakenings and daytime attacks of cataplexy and improve the structure of sleep in narcoleptic patients. The delta power and duration of slow wave sleep were increased and the frequency of transitions between wakefulness and REM sleep during the day was decreased under GHB treatment. In mice that lacked the GABA_{B1} subunit of GABA_B receptors, GHB failed to induce hypolocomotion, hypothermia, increase striatal dopamine synthesis, and induce delta-wave activity as compared to wild type animals [82]. Furthermore, signal transduction in brain membranes by GHB was blocked by a GABA_B receptor antagonist [83]. These are the clearest indications that GHB acts via GABA_B receptors. Nevertheless, the GHB radioligand-binding site appears to be different from GABA_B receptor-binding sites. The nature and relevance of the GHB binding sites remain elusive [82]. There are strong safety concerns for GHB in view of its potential for illicit use [84]. The cellular mechanisms underlying the dependence/abuse properties of GHB and its ability to induce absence seizures remain to be clarified [85, 86].

16.4 Pharmacology of GABA_B Receptors Beyond Narcolepsy

Although the presence of functional GABA_B receptors in the mammalian brain has been known for more than 20 years, there is still only one therapeutic agent in use, the muscle-relaxant baclofen, a GABA_B receptor agonist. Further potential indications for GABA_B receptor ligands are briefly summarized below [87].

Spasticity: The centrally mediated muscle-relaxant properties of baclofen are well established clinically, making baclofen the drug of choice in spasticity associated with cerebral palsy, multiple sclerosis, stiff-man syndrome and tetanus.

Pain: The antinociceptive action of baclofen is well established, an effect that is presumably due to a suppression of the neurotransmitter release from primary afferents (glutamate, substance P). However, rapid tolerance to this effect was also noted [87].

Cognition: Since baclofen is known to suppress cognitive behavior in animals, GABA_B receptor antagonists were developed as potential cognitive enhancers [88, 89]. CGP36742 (SGS 742) was tested in initial clinical trials to alleviate mild cognitive impairment.

Drug addiction: Baclofen was found to reduce the reinforcing effects of cocaine in rats at doses that did not affect locomotion. Other drugs of abuse such as nicotine, morphine, and ethanol were also sensitive to GABA_B receptor agonists. The clinical significance of these findings remains to be explored.

Depression and anxiety: Mice lacking GABA_{B1} or GABA_{B2} receptor subunits are more anxious but more resilient to stress (forced swim test) than controls.

The latter finding suggests that blockade of GABA_B receptors may produce antidepressant-like effects, while positive receptor modulators are anxiolytic [91].

Absence epilepsy: The site of origin of absence epilepsy is attributed to the somatosensory cortex as suggested by a genetic rat model of absence epilepsy. Neural excitation in this region spreads rapidly across the cortex and initiates a thalamocortical cascade. Injection of a GABA_B antagonist into the ventrobasal thalamus suppresses the spike and wave discharges [87].

In summary, the full therapeutic benefit of GABA_B receptor ligands has yet to be realized. Perhaps, the introduction of allosteric modulators – as a mechanistic analogy to benzodiazepines acting at GABA_A receptors – may bring about a new era for GABA_B receptor pharmacology.

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Development of Subtype-Selective GABA_A Receptor Compounds for the Treatment of Anxiety, Sleep Disorders and Epilepsy

John R. Attack

Abstract There is little doubt regarding the therapeutic possibilities of modulation of GABA_A receptor function as exemplified by the clinical utility of benzodiazepines for half a century. The emerging understanding of the role of different GABA_A receptor subtypes in mediating different physiological functions and pathological processes continues to offer opportunities for novel therapeutics. However, the challenge remains in turning the increased understanding of molecular pharmacology of GABA_A receptors into clinically efficacious drugs. Probably the most active area of research has been the search for a non-sedating anxiolytic that acts via the benzodiazepine binding site. Unfortunately, the clinical development of a number of drugs with promising pre-clinical profiles, such as ocinaplon, SL65.1498, pagoclone, MRK-409, TPA023 and TPA023B has halted for a variety of reasons. Therefore, the underlying hypothesis that sub-type-selective compounds are non-sedating anxiolytics in man remains to be adequately tested. As regards hypnotics, the benzodiazepine site compounds adiplon and indiplon, as well as the GABA_A $\alpha 4\beta\delta$ -preferring agonist gaboxadol, are no longer in clinical development, leaving EVT-201, which has demonstrated efficacy in Phase II studies in primary insomnia, as the single, novel GABA_A-mediated hypnotic currently under evaluation. Benzodiazepines remain the first-line treatment for status epilepticus, an indication for which, therefore, there would appear little need for GABA_A subtype-selective compounds. With respect to epilepsy, modulation of GABA_A receptor function via the neurosteroid recognition site is the mechanism of action of ganaxolone, which is currently being evaluated in Phase III studies in adult partial seizures and children with infantile seizures. The continually evolving understanding of the structure and function of not only the GABA_A receptor but also the variety of diverse binding sites that it harbours should continue to provide the molecular basis for designing strategies to selectively modulate the function of

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distinct subtypes of the GABA_A receptor and thereby provide novel therapies for the treatment of anxiety, sleep disorders, and possibly also epilepsy.

1 Introduction

1.1 GABA_A Receptor Structure and Function

The GABA_A receptor is a member of the Cys-loop ligand-gated ion channel family, which also includes the inhibitory glycine, ionotropic serotonin (5HT₃), and nicotinic acetylcholine receptors [1, 2]. The structural characteristics of these receptors are relatively well understood and is based upon homology modelling derived from the structure of the nicotinic acetylcholine receptor-binding protein [3, 4]. Hence, the Cys-loop family of receptors are homo- or heteropentameric assemblies of subunits arranged around a membrane-spanning pore. Each subunit has a large extra-cellular N-terminal domain, which contains the Cys-loop and is involved in agonist binding, as well as four membrane-spanning domains (TM1–4). The respective intracellular and extracellular loops between TMs 1–2 and 2–3 are relatively short whereas the intracellular loop between TMs 3 and 4 is comparatively long [1, 5], and the TM2 region is orientated such that it lines the ion channel pore (Fig. 1a; [6, 7]).

In the case of the GABA_A receptor, there are 16 related subunits (α 1–6, β 1–3, γ 1–3, δ , ϵ , θ , π) that comprise the “classical” GABA_A receptor plus an additional

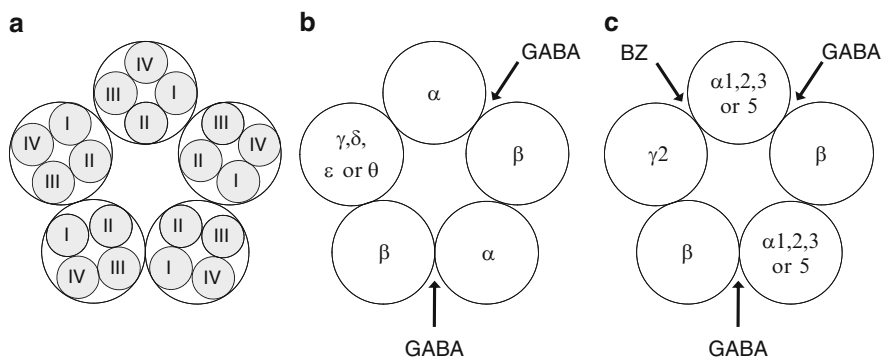


Fig. 1 Schematic representation of the arrangement of subunits in the GABA_A receptor as viewed from the synapse. **(a)** The GABA_A receptor is a heteropentameric arrangement of subunits, with each subunit containing four membrane-spanning regions (I–IV), of which the transmembrane region II lines the pore. **(b)** The most common arrangement of subunits is two α , two β and one γ , of which the latter may be replaced by either an δ , ϵ or θ subunit. The GABA binding site occurs at the interface of the α and β subunits. **(c)** When the γ subunit is $\gamma 2$ and the α subunit is either an $\alpha 1$, $\alpha 2$, $\alpha 3$ or $\alpha 5$ (but not $\alpha 4$ or $\alpha 6$), a benzodiazepine recognition site is formed at the interface of these subunits

three subunits (p1–3) that form the so-called GABA_C receptor [8–10]. The most common arrangement is α , β , and γ subunits in a 2:2:1 stoichiometry [11], although the γ subunit can be replaced by a δ , ε or θ subunit (Fig. 1b). The GABA recognition site occurs at the interface of the α and β subunits (Fig. 1b; [12]) and when a $\gamma 2$ subunit is adjacent to either an $\alpha 1$, $\alpha 2$, $\alpha 3$ or $\alpha 5$ subunit, a benzodiazepine recognition site is formed (Fig. 1c; [12]). Given the very large number of potential pentameric arrangements of the different GABA_A receptor subunits, it is perhaps surprising that to date only 26 subtypes fulfil the criteria for either “identified” (11 subtypes), “existence with high probability” (six subtypes) or “tentative” (nine subtypes) [10].

1.2 GABA_A Receptor Pharmacology

A number of different classes of pharmacological agents exert their effects on the GABA_A receptor by binding to recognition sites that are distinct from the endogenous ligand (GABA) binding site. Hence, barbiturates, ethanol, certain anaesthetics and convulsants as well as a variety of other compound classes act either via direct activation of the GABA_A receptor or allosteric modulation of the inhibitory effects of GABA [13, 14]. An understanding of how these various compounds interact with different subtypes of the GABA_A receptor forms the basis for developing compounds with novel pharmacological profiles that selectively interact with specific subtypes. In this regard, the benzodiazepine recognition site is the best understood based upon not only the proven clinical efficacy of compounds acting at this site but also the availability of pharmacological tool compounds as well as genetically modified mice. Accordingly, the focus of the subtype-selective GABA_A modulators described in the present article will be on compounds that act via the benzodiazepine binding site. Nevertheless, as the understanding of structure and pharmacology of the non-benzodiazepine binding sites increases (for example, the neurosteroid binding site [15–17]), it is probable that novel molecular targets on the GABA_A receptor will emerge. A brief overview of some of these different recognition sites is presented below.

1.2.1 GABA Binding Site

Based upon the structure of GABA, a number of structurally-related agonist or partial agonist analogues have been described (Fig. 2a) that are either conformationally restricted and/or contain a bioisosteric replacement of the carboxylic acid group found in GABA [18, 19]. These include the natural product muscimol, a constituent of the mushroom *Amanita muscaria*, which contains a 3-isoxazolol carboxylic acid bioisostere. Further conformational restriction of muscimol by incorporating the amino group of muscimol into a piperidine ring results in THIP

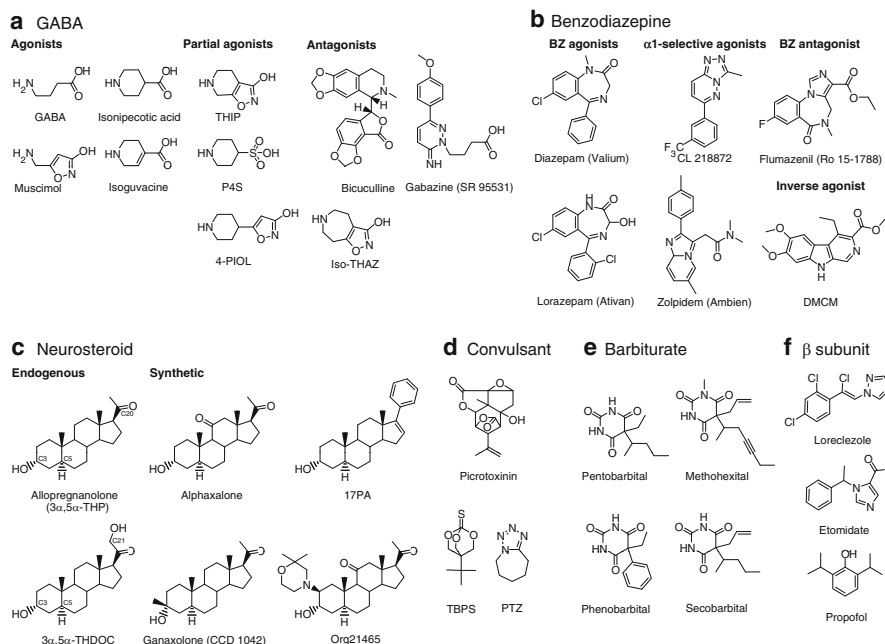


Fig. 2 Structures of different classes of compounds known to bind to the various recognition sites associated with the GABA_A receptor. **(a)** At the agonist (GABA) binding site, the endogenous ligand GABA and its structural analogue muscimol acts as agonists whereas bicuculline is the prototypic antagonist. **(b)** The 1,4-benzodiazepines as well as the non-benzodiazepine, $\alpha 1$ subtype-preferring agonists CL 218872 and zolpidem bind to the benzodiazepine recognition site. At this same site, flumazenil (Ro 15-1788) and DMCM are prototypic antagonists and inverse agonists, respectively. **(c)** At the neurosteroid binding site, allopregnanolone and $3\alpha,5\alpha$ -THDOC are endogenous ligands and a number of structurally related synthetic ligands have also been described, of which alphaxalone and ganaxalone are most noteworthy since the former is the active component of the anaesthetic Althesin and the latter is currently undergoing clinical trials as a treatment for epilepsy. **(d)** Picrotoxinin, TBPS and PTZ bind within the ion channel pore itself and in doing so block GABA-mediated inhibitory transmission thereby causing seizures, and consequently, this recognition site is known as the convulsant binding site. **(e)** Numerous barbiturates have been described, which are all based upon the structure of barbituric acid. The recognition site for these compounds is less well described than other binding sites on the GABA_A receptor. **(f)** The anti-convulsant loreclezole and the anaesthetics etomidate and propofol bind to recognition sites associated with the β subunit. *Abbreviations:* 17PA $3\alpha,5\alpha$ -17-phenylandrosterone-16-en-3-ol; $3\alpha,5\alpha$ -THDOC $3\alpha,5\alpha$ -tetrahydrodeoxycorticosterone; 4-PIOL 5-(4-piperidyl)isoxazole-3-ol; DMCM methyl-6,7-dimethoxy-4-ethyl- β -carboline-3-carboxylate; iso-THAZ 5,6,7,8-tetrahydro-4H-isoxazolo[3,4-d]azepin-3-ol; P4S piperidine-4-sulphonic acid; PTZ pentylenetetrazole; TBPS *t*-butylbicyclophosphorothionate; THIP gaboxadol, 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol

[20], which has subsequently become known as gaboxadol. By analogy with THIP, the incorporation of the amino group in GABA into a piperidine was used to produce the monoheterocyclic agonist, isonipecotic acid, and the introduction of a double bond yielded isoguvacine. Replacement of the carboxylic acid group of

isonipecotic acid resulted in compounds such as P4S and 4-PIOL. As regards GABA site antagonists, the prototypic compound is the alkaloid bicuculline [21]. However, additional structural classes of antagonists include the arylaminopyridazine analogues of GABA, typified by Gabazine (SR 95531; [22]) and iso-THAZ, a bicyclic 5-isoxazolo analogue of THIP (Fig. 2a).

Despite the variety of possible combinations of α 1–6 and β 1–3 subunits that could theoretically comprise the GABA recognition site, most full agonists, such as GABA, muscimol and isoguvacine, and antagonists, for example, bicuculline and Gabazine, demonstrate little difference in affinity between recombinant GABA_A receptor sub-types [18, 19, 23, 24]. Moreover, the very defined requirements for binding at the GABA recognition site mean that relatively few different structural classes of compound binding at this site have been reported. In contrast to full agonists and antagonists, certain partial agonists appear to have a degree of subtype selectivity. Such compounds include 4-PIOL, P4S and THIP [18, 19] and whereas non-selective GABA site agonists and antagonists would appear to be of limited therapeutic potential, partial agonists that possess a degree of subtype-selectivity, such as THIP (Gaboxadol), may be of therapeutic benefit, for example, in sleep disorders (see below). A 3D-pharmacophore model for the GABA binding site has been described [25, 26].

1.2.2 Benzodiazepine Binding Site

Since their introduction half a century ago [27], the therapeutic utility of benzodiazepines as hypnotics, anticonvulsants, muscle relaxants [28, 29] and, particularly, anxiolytics [30] has become well established. However, the choice of particular benzodiazepines as, for example, either anxiolytics or hypnotics, is related more to pharmaceutical and pharmacokinetic properties and commercial considerations than to intrinsic differences in pharmacology [31]. With respect to pharmacokinetics, benzodiazepines can be categorised as short-, intermediate- and long-acting in a manner analogous to the classification of barbiturates as short-intermediate- and long-acting that dictated their use as anaesthetics, hypnotics and anxiolytics, respectively. The prototypic benzodiazepine is diazepam (Fig. 2b), which is more specifically described as a 1,4-benzodiazepine referring to the position of the nitrogens in the diazepine core. Multiple other analogues of diazepam have been used in the clinical, of which lorazepam (Fig. 2b) is but one example.

In addition to their clinical efficacy, benzodiazepines are a particularly attractive class of drugs since they have low levels of toxicity, especially compared to the barbiturates, which they superseded in clinical practice [32, 33]. The safety of benzodiazepines is based primarily upon the fact that they cannot directly activate the GABA_A receptor; rather, they modulate the inhibitory functions of GABA by allosterically increasing the frequency of GABA-induced channel opening events. Such compounds are described as benzodiazepine site agonists (or positive

allosteric modulators) whereas those that reduce GABA-mediated chloride flux are known as inverse agonists (negative allosteric modulators). Compounds such as flumazenil, which bind with high affinity but do not affect GABA-induced chloride currents, exert no physiological effect on the GABA_A receptor but can block, or antagonise, the effects of benzodiazepine site agonists or inverse agonists and are therefore described as benzodiazepine site antagonists.

Non-benzodiazepine chemical structures also bind to this recognition site and the differential affinity of the triazolopyridazine CL 218872 (Fig. 2b) for GABA_A receptors in different parts of the brain was the initial indication for the heterogeneity of benzodiazepine binding sites [34]. The advent of molecular cloning and the detailed pharmacological characterisation of recombinant receptors further refined the heterogeneity of GABA_A subtypes [13]. It is now apparent that the benzodiazepine binding site occurs at the interface of the α and $\gamma 2$ subunits of GABA_A receptors with either an $\alpha 1\beta\gamma 2$, $\alpha 2\beta\gamma 2$, $\alpha 3\beta\gamma 2$ or $\alpha 5\beta\gamma 2$ subunit composition [35], a combined GABA_A receptor population that accounts for around 75% of total brain GABA_A receptors [36, 37]. Most notably, receptors with an $\alpha 4\beta\gamma 2$ or $\alpha 6\beta\gamma 2$ composition have no affinity for classical benzodiazepines such as diazepam, and this can be attributed to a single amino acid, which is a histidine in $\alpha 1$, $\alpha 2$, $\alpha 3$ and $\alpha 5$ subunits, but an arginine in $\alpha 4$ and $\alpha 6$ subunits [38].

The fact that the single histidine to arginine switch in the α subunit confers diazepam insensitivity to the GABA_A [38] has been exploited to generate a number of point-mutated mice in which particular populations of GABA_A receptor (i.e. $\alpha 1$ -, $\alpha 2$ -, $\alpha 3$ -, or $\alpha 5$ -containing receptors) retain their normal physiological GABA-gated chloride flux function but are insensitive to the pharmacological effects of diazepam [39]. Using this strategy, Mohler, Rudolph and colleagues plus, to a lesser extent, the group at Merck have begun to dissect which of the pharmacological features of diazepam in particular, but also certain other benzodiazepine site modulators, are associated with which specific subtypes of GABA_A receptor [40–46]. These studies have been complemented by observations in α subunit-deleted (knock-out) mice as well as the use of GABA_A subtype-selective pharmacological tool compounds. Collectively, these data show that the $\alpha 1$ subtype is associated with sedation whereas the $\alpha 2$ and/or $\alpha 3$ subtypes are the “anxiolytic” subtypes [40, 41, 47, 48] and the $\alpha 5$ subtype is associated with aspects of cognition [49–54]. Although the delineation of GABA_A subtypes into $\alpha 1$ = sedation, $\alpha 2/\alpha 3$ = anxiety, and $\alpha 5$ = cognition is undoubtedly a gross over-simplification, it does nevertheless form the basis for the hypotheses that compounds that have (1) preferential agonist activity at the $\alpha 1$ subtype should be sedative-hypnotics; (2) agonist or partial agonist activity at the $\alpha 2$ and $\alpha 3$ subtypes but have reduced or, preferably, no activity at the $\alpha 1$ subtype should be non-sedating anxiolytics [55, 56]; and (3) $\alpha 5$ -selective inverse agonism should improve cognitive function [52]. There is already a degree of clinical validation for the hypothesis that the $\alpha 1$ subtype is associated with sedation in so far as the sedative-hypnotic zolpidem (Ambien) is $\alpha 1$ subtype-preferring (see below). As regards anticonvulsant activity, there is no specific “anticonvulsant subtype”, with data suggesting that efficacy at

more than one subtype is required, with the $\alpha 3$ and $\alpha 5$ subtypes possibly playing a lesser role [44].

1.2.3 Neurosteroid Binding Site

Neuroactive steroids (Fig. 2c) are endogenous neuromodulators that are synthesised both in the brain as well as in the adrenal glands, ovaries and testes [57, 58]. The recognition that they are potent modulators of GABA_A receptor function was based upon observations with alphaxalone (3 α -hydroxy-5 α -pregnan-11,20-dione; [59]), a component of the steroid anaesthetic Althesin, which was introduced for clinical use as an anaesthetic in Europe in the 1970s but was withdrawn due to adverse events associated with the vehicle in 1985 [60]. These observations were extended to include the naturally occurring neurosteroids allopregnanolone (3 α -hydroxy-5 α -pregnan-20-one or 3 α ,5 α -THP) and pregnanolone (3 α -hydroxy-5 β -pregnan-20-one or 3 α ,5 β -THP), both of which also enhanced GABAergic neurotransmission [61]. Additional endogenous neurosteroids that interact with the GABA_A receptor include a variety of 3 α ,5 α - and 3 α ,5 β -reduced metabolites of deoxycorticosterone, dihydroepiandrosterone and testosterone [57, 58].

At relatively low concentrations (<1 μ M), neurosteroids act by increasing both the frequency and the duration of GABA-induced channel opening events, whereas at higher concentrations (>1 μ M), neurosteroids, like barbiturates, can directly activate the GABA_A receptor [62, 63]. These data suggest that there may be different recognition sites which mediate the potentiating and direct activating actions of neurosteroids. This has been confirmed by using structural homology modelling plus chimaeras of mouse $\alpha 1$ and $\beta 2$ subunits and the neurosteroid-insensitive *Drosophila Rdl* (resistance to dieldrin) GABA receptor. Along with mutagenesis experiments, these studies have identified the $\alpha 1$ subunit TM1 Q241 as well as TM3 N407 and Y410 residues as crucial for conferring sensitivity to the modulatory aspect of neurosteroid function, whereas there is a separate binding site formed by the TM1 α T236 and TM3 β Y284 residues that mediates the direct activation of neurosteroids [15–17]. Furthermore, there may also be one or possibly two additional binding sites on mammalian GABA_A receptors associated with the inhibitory sulphated steroids [16]. Although neurosteroids modulate most GABA_A receptor isoforms, they have a modestly higher affinity for the $\alpha\beta\delta$ -containing subtypes [63–65]. However, the δ subunit does not appear to contribute to the binding site but is more likely associated with the degree of potentiation [15–17].

The realisation that endogenous and synthetic neurosteroids acted via the GABA_A receptor triggered the search for additional synthetic neurosteroids based upon, for example, tricyclic analogues of steroids and unnatural enantiomers of endogenous neuroactive steroids [60, 66, 67]. More recently, 17PA (3 α ,5 α -17-phenylandroster-16-en-3-ol) has been described as a neurosteroid antagonist that does not affect GABA-mediated responses but does block the effects of 3 α ,5 α -THP (but not, interestingly 3 α ,5 β -THP) [68]. Pre-clinical data indicate that synthetic neurosteroids might be of therapeutic utility based upon their anxiolytic,

anaesthetic, hypnotic, antimigraine and anticonvulsant properties [67], although this potential has yet to be fulfilled. For example, despite initial clinical studies being promising, a double-blind, placebo-controlled Phase II study of a tablet formulation of ganaxolone did not show significant efficacy in acute migraine [67], although this may have been a consequence of the slow T_{\max} for this compound. In addition, the development of the water-soluble neurosteroid anaesthetic Org21465 was discontinued in 1996 due to side-effects [67]. Furthermore, CoCensys (now Purdue Neuroscience) selected the neurosteroids CCD-3693 [69] and Co 2-6749 (also known as GMA-839 or WAY-141839; [70]) for clinical development as a hypnotic and anxiolytic, respectively [67]. However, by the beginning of 2003, development of the former was reported to have been halted, whereas there has been no update on the development of Co 2-6749 since 1999 [67]. Nevertheless, ganaxolone (CCD 1042; [71]) is currently under evaluation for uncontrolled adult partial seizures and infantile spasms (see below).

The role of different GABA_A receptors in mediating not only the physiological functions of endogenous neurosteroids but also the pharmacological effects mediated via this site (which include anxiolytic, anaesthetic, hypnotic and anticonvulsant properties; [67]) remains poorly understood, and it is therefore difficult to predict the possible therapeutic benefits of selectively modulating GABA_A receptor subtypes via the neurosteroid recognition site. Nevertheless, the identification of specific neurosteroid recognition sites [15–17] should permit the generation of, for example, α subunit Q241 point-mutated neurosteroid-insensitive knock-in mice [72, 73] that will help elucidate the role of specific GABA_A receptor subtypes in mediating the different functions of neurosteroids in a manner analogous to the methods used to dissect the subtype-selective functions of benzodiazepines and anaesthetics [43, 74]. Moreover, the identification of the different binding sites that mediate the modulatory and direct activation aspects of neurosteroid function may provide the basis for identifying compounds that selectively modulate, but do not directly activate, GABA_A receptors. Such compounds might therefore be expected to have an improved therapeutic index relative to neurosteroids that not only modulate but also directly activate the receptor.

1.2.4 Convulsant Binding Site

A number of structurally unrelated compounds bind to the GABA_A receptor and block GABA-gated chloride flux in a non-competitive manner, resulting in convulsions in animals [75]. Compounds that bind to this convulsant binding site (Fig. 1d) include picrotoxinin (the active component of picrotoxin), pentylenetetrazole, a number of insecticides (including dieldrine) and TBPS. This latter compound can be radiolabelled and the modulation of [³⁵S]TBPS or, alternatively, [³H]TBOB or [³H]EBOB binding can be used as an indicator of the efficacy of compounds that allosterically modulate GABA_A receptor function [76, 77]. However, given that compounds that bind at this site produce convulsions, there is little apparent therapeutic benefit for drugs acting at this particular site on the GABA_A

receptor. Nevertheless, sub-convulsant doses of picrotoxin and pentylenetetrazole have been described as alleviating the cognitive deficit associated with a mouse model of Down syndrome [78]. Although there does appear a degree of heterogeneity in the agonist-induced modulation of TBPS binding to the convulsant site of different GABA_A receptor populations [79–81], it is unclear how this might be exploited to pharmacological advantage.

1.2.5 Barbiturate Binding Site

Although barbituric acid does not have any effects on the CNS, the barbiturates that derive from it have a pronounced pharmacology. The initial barbiturate, diethylbarbituric acid, was synthesised in 1903 and was made by substituting two hydrogens for ethyl groups at the same C-5 position. This compound, barbital (Veronal; Fig. 1e), was found to be hypnotic and has a relatively long half-life. Subsequently, phenobarbital (Luminal) was marketed as a sedative and anticonvulsant, and thereafter, additional barbiturates were introduced, including amobarbital (Amytal), pentobarbital (Nembutal), secobarbital (Seconal) and the ultra short-acting hexobarbital (Evipal), thiopental (Pentothal) and methohexital (Brevital). The clinical use of barbiturates is dictated to a large extent by their respective plasma half-lives. Hence, ultra-short to short-acting barbiturates are used for anaesthesia, the short-intermediate acting barbiturates may be used for insomnia and/or anxiety and the long-acting barbiturates, such as phenobarbital, have utility as anticonvulsants, particularly as a second line treatment for status epilepticus. Despite the decline in the use of barbiturates as hypnotics and anxiolytics, they have retained their clinical utility as anaesthetics and anticonvulsants, with thiopental and phenobarbital being respective examples [82, 83].

Barbiturates exert their effects via GABA_A-mediated inhibitory neurotransmission [84–86] with, for example, their functional effects at the GABA_A receptor correlating with their anaesthetic potency [87]. More specifically, at lower concentrations (below ~0.1 mM), barbiturates modulate the effects of GABA, but at intermediate concentrations (~0.1–1 mM), they directly activate the GABA_A receptor, and at high concentrations (>1 mM), they can block the ion channel [88, 89]. Although the barbiturate binding site(s) remains to be fully defined, it does appear to be associated with the β subunit [90, 91], and the allosteric changes produced by direct activation of the GABA_A by pentobarbital are distinct from those produced by GABA [89].

1.2.6 β Subunit Binding Site(s)

The broad spectrum anticonvulsant loreclezole was shown to selectively activate β 2- and β 3-, but not β 1-containing GABA_A receptors [92]. This selectivity can be attributed to a single amino acid in the TM2 region of the β subunit, which is a serine in the β 1 subunit and an arginine in the β 2 and β 3 subunits [93]. Similar data were observed using etomidate [94–96], which although used clinically as an intravenous anaesthetic has a structural resemblance to loreclezole (Fig. 2f).

In a manner analogous to the use of α subunit point-mutated mice to identify GABA_A subtypes associated with the various pharmacological properties of benzodiazepines [43], mice containing point mutations of the β subunit, e.g., $\beta 2$ (Asn265Ser) and $\beta 3$ (Asn265Met), have been used to attribute distinct aspects of the in vivo profile of β subunit-interacting compounds to specific GABA_A subtypes. Such compounds include the intravenous anaesthetics etomidate and propofol [97–99], the anticonvulsant loreclezole [100], the general anaesthetic isoflurane [101] and the anaesthetic barbiturate pentobarbital [91].

Based upon the selectivity of enaminones [102] for $\beta 2$ - and $\beta 3$ - over $\beta 1$ -containing GABA_A receptors, it has been proposed that such compounds might be a novel way of developing anxiolytic compounds [103]. Although this is still an emerging area, it nevertheless highlights the possibility of developing GABA_A receptor subtype-selective compounds via mechanisms distinct from the benzodiazepine binding site.

1.3 Subtype-Selective Affinity and Subtype-Selective Efficacy

In the search for GABA_A receptor subtype-selective compounds, two different approaches may be employed, namely subtype-selective affinity and subtype-selective efficacy. The selective affinity strategy is probably the most intuitive and describes a compound that binds preferentially to the subtype of interest with much lower, or preferably negligible, affinity at the other subtypes (Fig. 3a). Although such a compound might possess equivalent efficacy at each of the subtypes to which it binds, particular the in vivo effects will be mediated predominantly via the high-affinity subtypes since the compound will preferentially occupy these receptors in vivo. The alternative approach is to design compounds with subtype-selective efficacy (Fig. 3b). Such compounds bind with equivalent affinity to all four subtypes but only have efficacy at certain of those receptors. The antagonist efficacy at other subtypes means that even though the compound might bind with very high affinity, there is no consequence for GABA-mediated receptor function. Although the difference between the selective affinity and selective efficacy approaches are illustrated (Fig. 3) in terms of the benzodiazepine recognition site (i.e. $\alpha 1$ -, $\alpha 2$ -, $\alpha 3$ - and $\alpha 5$ -containing GABA_A subtypes), the principle is equally applicable to other binding sites on the GABA_A receptor.

2 GABA_A Receptor Compounds for Anxiety

2.1 Anxiety Disorders and Benzodiazepines

Anxiety disorders are a group of conditions [30] that include social anxiety disorder (also known as social phobia; which has an estimated lifetime prevalence in the region of 13%), phobias in general (11%), post-traumatic stress disorder (8%), generalised

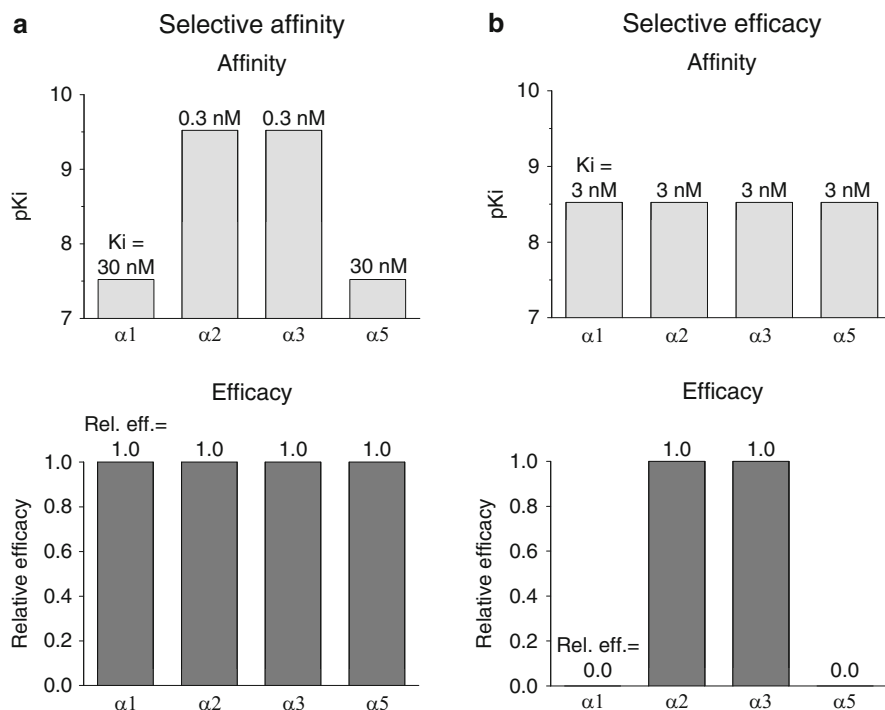


Fig. 3 Schematic representation of compounds with either subtype-selective affinity or subtype-selective efficacy for the benzodiazepine site of different GABA_A receptors. **(a)** A compound with subtype-selectivity affinity binds to the benzodiazepine binding site of the GABA_A receptor subtypes with differing affinities (in this example a 100-fold higher affinity for $\alpha 2$ and $\alpha 3$ versus $\alpha 1$ and $\alpha 5$ subtypes), yet when bound, the compound has equivalent (in this case full agonist) efficacy at each subtype, albeit with a 100-fold difference in the EC₅₀ for agonist efficacy at the $\alpha 2$ and $\alpha 3$ versus $\alpha 1$ and $\alpha 5$ subtypes. **(b)** In contrast, a compound with subtype-selectivity efficacy binds to the benzodiazepine binding site of the GABA_A receptor subtypes with equivalent affinity (in this example 3 nM), but the compound has differential efficacy at the various subtypes (in this case full agonist efficacy at the $\alpha 2$ and $\alpha 3$ subtypes and antagonist efficacy at the $\alpha 1$ and $\alpha 5$ subtypes). It is important to note that these are hypothetical examples since in practise it has not proved possible to achieve the levels of $\alpha 2/\alpha 3$ binding selectivity illustrated in *panel A* (with a maximal, approximately tenfold binding $\alpha 3$ versus $\alpha 1$ selectivity having been reported; [104]). Similarly, it has not been possible to demonstrate the level of efficacy selectivity illustrated in *panel B* with at best partial, or weak partial agonist efficacy at the $\alpha 2$ and $\alpha 3$ and antagonist efficacy at the $\alpha 1$ and $\alpha 5$ subtypes being described [104]

anxiety disorder (GAD; 5%), panic disorder (4%) and obsessive-compulsive disorder (2%). In the USA alone, the estimated total annual cost of anxiety disorders (including direct and indirect costs) is in the region of sixty billion dollars [30]. Although a variety of drugs are used to treat these disorders, the most common ones are antidepressants, particularly SSRIs and the 5HT_{1A} partial agonist buspirone, and benzodiazepines [30, 105–107]. Despite the undoubted anxiolytic efficacy of

benzodiazepines, major concerns related to the use of this class of drugs include abuse, dependence and withdrawal [33, 108–111]. In conjunction with the over-prescribing of benzodiazepines that occurred in the 1960s and 1970s, a negative public perception of this class of drugs developed [29, 112]; a perception that lingers to this day [107]. An additional liability associated with benzodiazepine use in the treatment of anxiety disorders is that of sedation which, although a desirable pharmacological effect when benzodiazepines are used as hypnotics, is clearly undesirable in the performance of activities of daily living, such as operating machinery or driving [113] and is also a risk factor associated with falls in the elderly [114].

Based upon the proven clinical efficacy and safety of benzodiazepines, as well as the pre-clinical rationale emerging from the use of transgenic mouse models and the extensive understanding of the benzodiazepine recognition site, efforts to develop the next generation of anxiolytic GABA_A modulators have primarily focused upon compounds that modulate GABA_A receptor function via the benzodiazepine binding site. More specifically, the hypothesis that GABA_A modulators that modulate the $\alpha 2$ and $\alpha 3$ subtypes to a greater extent than $\alpha 1$ -containing GABA_A receptors, should be anxiolytic but with a reduced sedation liability [115], has resulted in the identification of a number of $\alpha 2/\alpha 3$ -selective compounds that are either pre-clinical tool compounds or clinical development candidates, examples of which are discussed below.

2.2 Benzodiazepine Site Modulators: Pre-clinical Compounds

A number of subtype-selective benzodiazepine site GABA_A modulators have been reported to be non-sedating anxiolytics in pre-clinical species. These include the prototypic $\alpha 2/\alpha 3$ efficacy-selective triazolopyridazine L-838417 [42, 116], which has partial agonist efficacy at the $\alpha 2$, $\alpha 3$ and $\alpha 5$ subtypes but, most notably, has antagonist efficacy at $\alpha 1$ -containing GABA_A receptors (Fig. 4a). This compound proved to be anxiolytic, but with a much reduced sedation liability in both rodent and primate species [42, 116]. However, pharmacokinetic issues restricted its use to pre-clinical species [118]. The Merck group also described the fluoroimidazopyridine TP003 (Fig. 4b; [48]) as an $\alpha 3$ -selective partial agonist, and as such, it is one of the few examples where there is a marked separation of $\alpha 2$ and $\alpha 3$ efficacy since, generally speaking, the efficacy of a compound at these two subtypes is comparable [104]. Like L-838417, TP003 was also anxiolytic in rodent and primate species and provides support for the notion that the $\alpha 3$ subtype is an important contributor to the anxiolytic properties of non-selective benzodiazepines [48]. Unfortunately, poor pharmacokinetic characteristics once again prevented this compound being developed further. MRK-529 (Fig. 4c) is an $\alpha 2/\alpha 3$ efficacy-selective tricyclic pyridone, which has a non-sedating anxiolytic profile as well as favourable pharmacokinetic properties in pre-clinical species. However, progression of this compound into clinical studies was halted due to phototoxicity in pre-clinical safety and toxicity studies [104]. The Danish company NeuroSearch have described

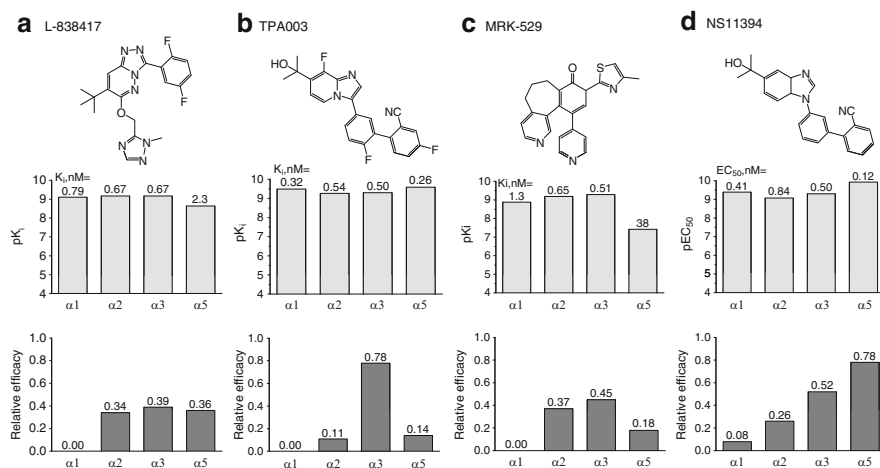


Fig. 4 Examples of the structure and in vitro binding and relative efficacy profiles of compounds that are non-sedating anxiolytics in pre-clinical species but did not progress into clinical development. For each compound, the upper panel represents the affinity, plotted as the pK_i or, where data are derived from functional assays, pEC_{50} (with values above bars showing the mean affinity expressed in nM). Efficacy data are expressed relative to a non-selective benzodiazepine agonist. Data are derived from: (a) L-838417, [42]; (b) TP003, [48]; (c) MRK-529, [104]; (d) NS11394, [117]

NS11394 (Fig. 4d) as a benzimidazole, which is structurally related to NS2710, a compound that was in Phase II clinical studies but whose development was halted due to skin rash [115]. This more recent compound was, like L-838417, selective for the $\alpha 2$ and $\alpha 3$ subtypes and was a non-sedating anxiolytic in rodents [117]. Moreover, this compound also possessed efficacy in a number of pre-clinical pain models [119].

Importantly, these compounds collectively demonstrate that not only can $\alpha 2/\alpha 3$ -selective efficacy be established with a variety of different chemotypes (in this case using triazolopyridazine, fluoroimidazopyridine, pyridone and benzimidazole scaffolds) but also that such compounds consistently behave as non-sedating anxiolytics in pre-clinical species. Moreover, $\alpha 2/\alpha 3$ -selective compounds have also been reported in a variety of other structural classes, including imidazopyrimidine, imidazotriazine, imidazopyrazinone, pyrazolotriazine, pyridazine and pyrazolopyridinone [56, 104].

2.3 Benzodiazepine Site Modulators: Clinical Compounds

Although the $\alpha 2/\alpha 3$ -selective compounds described in the previous section clearly highlight the non-sedating anxiolytic behaviour of such compounds in pre-clinical

species, the critical issue is the translation of the pre-clinical pharmacology profile into clinical utility. The difficulty in making this transition is best exemplified by the non-selective partial agonist bretazenil, which despite demonstrating a clear separation between anxiolytic and sedating doses in pre-clinical studies did not show the corresponding separation in man [120]. Although the primary focus is to demonstrate a non-sedating anxiolytic profile in man, consideration should also be given to the abuse potential of such compounds since the scheduling (or ideally non-scheduling) of a drug for an indication such as GAD has a marked influence on the prescribing habits of doctors. In the present section, the fate of a variety of compounds that act at the benzodiazepine binding site (some of which are not particularly $\alpha 2/\alpha 3$ subtype-selective) and progressed into clinical development are summarised.

2.3.1 Ocina-plon

Ocina-plon (also known as CL 273,547; [121, 275]) is a pyrazolopyrimidine (Fig. 5a) that is structurally related to zaleplon (Sonata®, CL 284,846) and indiplon (see below). It has low affinity for GABA_A receptors ($IC_{50} > 5 \mu M$ at each subtype) but has higher efficacy at the $\alpha 1$ compared to $\alpha 2$, $\alpha 3$ and $\alpha 5$ subtypes [121, 128]. However, despite its high efficacy at the “sedation” ($\alpha 1$) GABA_A subtype, it has an anxiolytic profile in pre-clinical species with, for example, an approximately 30-fold difference between the doses that produced anxiolytic-like activity and sedation in rhesus monkey [121, 129]. In a short, 2-week, 3-arm (placebo and 180 or 240 mg/day ocina-plon; $n = 31$ –35/group), proof-of-concept study in GAD, ocina-plon produced a significantly greater reduction in the Hamilton Anxiety (HAM-A) scale when compared to placebo-treated subjects at a dose of 240 mg/day (120 mg twice a day) without significant signs of sedation [121]. This was followed up by a 4-week, 2-arm study (placebo and 270 mg ocina-plon – 90 mg three times a day, $n = 29$ and 31, respectively), which again demonstrated that ocina-plon was more effective than placebo at reducing the HAM-A score, albeit with the caveat that out of the 60 subjects initially assigned to a treatment group, only 23 completed the entire 4 weeks since the study was terminated early due to a serious adverse event that was considered to be possibly drug-related [130]. More specifically, a single subject had a significant elevation of serum liver alanine and aspartate transaminase enzymes, with a peak of >50-fold higher than the upper limit of normal followed by icterus (jaundice). Nevertheless, and as in the initial proof-of-concept study, ocina-plon did not demonstrate signs of sedation. However, as the Phase III study of ocina-plon had to be halted, development was discontinued at the end of 2005.

Clearly, the behaviour of ocina-plon as a non-sedating anxiolytic is at odds with the prevailing $\alpha 1 =$ sedation hypothesis and the reason for its lack of sedation in pre-clinical species and in man remain uncertain [121, 129, 130]. Furthermore, DOV 51892, which is structurally related to ocina-plon, is essentially a “super-agonist”

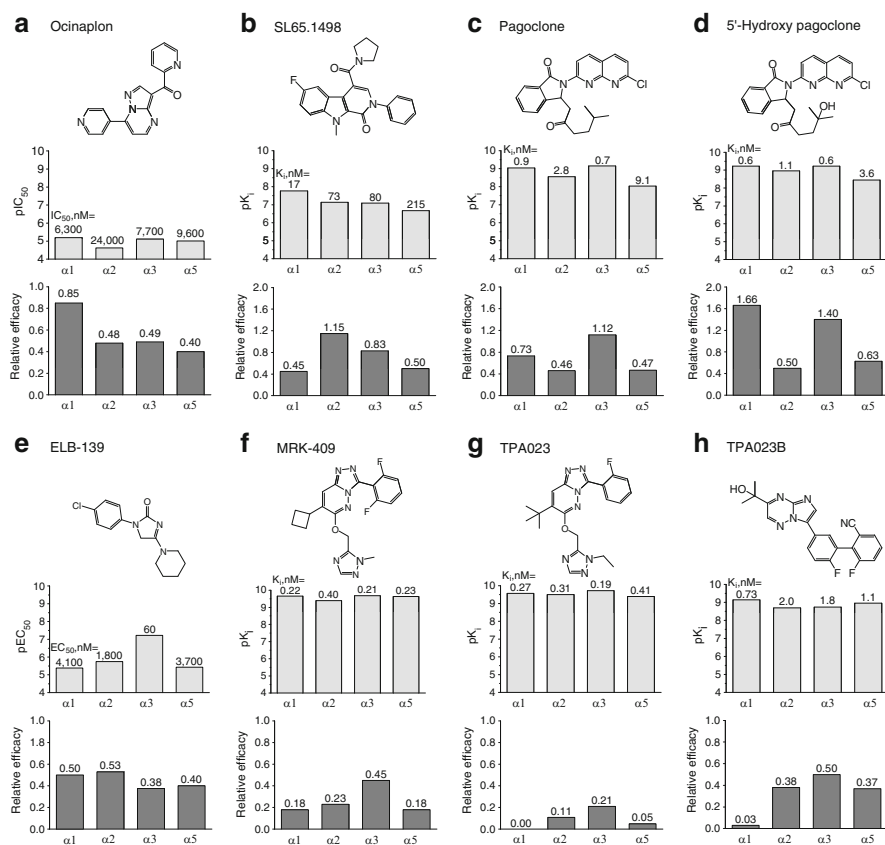


Fig. 5 The structure and in vitro binding and relative efficacy profiles of compounds that modulate GABA_A receptor function via the benzodiazepine binding site, are non-sedating anxiolytics in pre-clinical species and have progressed into clinical development. For each compound, the upper panel represents the affinity, plotted as the pK_i , pEC_{50} or, where data are derived from functional assays, pEC_{50} (with values above bars showing the mean affinity expressed in nM). Efficacy data are expressed relative to a non-selective benzodiazepine agonist. Data are derived from: (a) Ocinalopn, [121]; (b) SL65.1498, [115, 122]; (c and d). Pagoclone and 5'-hydroxy pagoclone, [123]; (e) ELB-139, [124]; (f) MRK-409, [125]; (g) TPA023, [126]; (h) TPA023B, [127]

at the $\alpha 1$ subtype yet pre-clinically possesses a non-sedating anxiolytic profile [131], suggesting that, and for whatever reason, efficacy at the $\alpha 1$ subtype may not per se be a reliable indicator of sedation liability in man [132].

2.3.2 SL65.1498

Sanofi-Synthelabo (now Sanofi-Aventis) described SL65.1498 as a pyridoindole with full agonist efficacy at the $\alpha 2$ and $\alpha 3$ subtypes and lower efficacy at the $\alpha 1$

and $\alpha 5$ subtypes (respective relative efficacy values of 1.15, 0.83, 0.45 and 0.50; Fig. 5b). However, the $\alpha 2/\alpha 3$ versus $\alpha 1$ -selective efficacy is offset, to a certain extent, by the approximately fivefold higher affinity of SL65.1498 for $\alpha 1$ - compared to $\alpha 2$ - and $\alpha 3$ -containing GABA_A receptors. Nevertheless, in a pharmacodynamic study in normal volunteers, SL65.1498 did not show overt signs of sedation even at a dose of 25 mg, despite possessing significant $\alpha 1$ efficacy and a slight $\alpha 1$ binding selectivity [133]. Moreover, although SL65.1498 did decrease saccadic peak velocity at a dose of 25 mg (but not 2.5 or 7.5 mg), the extent of this reduction was much less than that observed not only with lorazepam [133] but also TPA023 and MRK-409 [134, 135]. Although the maximum dose used for SL65.1498 (25 mg) was much higher than that used for either TPA023 or MRK-409 (1.5 mg and 0.75 mg, respectively; [134, 135]), this is probably related more to the lower affinity of SL65.1498 at the $\alpha 1$, $\alpha 2$ and $\alpha 3$ subtypes (17–80 nM; [122]) compared to either TPA023 (K_i values ranging from 0.19–0.41 nM and 0.21–0.40 nM, respectively) rather than reflecting differences in tolerability. SL65.1498 was described in February of 2004 as undergoing Phase IIb studies for the indications of anxiety and muscular spasms. However, following the merger of Sanofi-Synthelabo and Aventis in August 2004, the drug did not appear in the March 2005 pipeline of the newly formed Sanofi-Aventis pipeline.

2.3.3 Pagoclone

Pagoclone is a member of the cyclopyrrolone series (Fig. 5c), that also includes suriclone and zopiclone [136]. It is the active enantiomer of the racemate RP 59037 [137], which was originally described as possessing partial agonist efficacy [138] and an anxiolytic profile in pre-clinical animal models [139], although others have reported little separation between anxiolytic and sedative doses in rat [123]. It has equivalent affinity at the $\alpha 1$, $\alpha 2$ and $\alpha 3$ subtypes with 3–10-fold lower affinity at the $\alpha 5$ subtype (Fig. 5c). The active metabolite, 5'-hydroxypagoclone, has a similar binding profile compared to its parent but has increased efficacy at $\alpha 1$ -containing receptors (Fig. 5d).

In man, a dose of 0.4 mg pagoclone resulted in GABA_A receptor occupancy, as measured using [¹¹C]flumazenil positron emission tomography (PET), of 15% whereas 1 mg lorazepam produced 6% occupancy. However, despite its greater occupancy, pagoclone produced changes in saccadic eye movements that were comparable to lorazepam, suggesting that it behaves in man as a partial agonist [140]. Consistent with this, pagoclone produced relatively mild and transient effects on learning and memory at doses of 0.15, 0.3 and 0.6 mg [141]. On the other hand, in a comparative study in healthy recreational drug users, pagoclone (4.8 mg) was rated as being similar to diazepam [142], suggesting that its efficacy profile may not be too dissimilar from that of a non-selective full agonist and that the abuse potential of pagoclone might be comparable to that of diazepam.

Pagoclonone was originally licensed from Rhone-Poulenc Rorer (which became Aventis and then Sanofi-Aventis) to Interneuron (which became Indevus and then Endo Pharmaceuticals) and was being co-developed by Warner-Lambert (which became part of Pfizer) for the treatment of panic attacks and GAD [136, 143, 144]. However, development for these disorders was discontinued due to lack of robust efficacy [55]. Nevertheless, pagoclonone is still being evaluated as a treatment for stuttering. Hence, an initial 8-week, placebo controlled, double-blind, multi-centre Examining Pagoclonone for Persistent Developmental Stuttering Study (EXPRESS) study demonstrated proof-of-concept in developmental stuttering in patients given escalating doses of pagoclonone from 0.3 to 0.6 mg/day. As a result, Teva Pharmaceutical, in collaboration with Endo, has evaluated pagoclonone in additional Phase II studies of developmental and adult stuttering (www.ClinicalTrials.gov, identifiers NCT00830154 and NCT00239915). The drug has also been evaluated as a potential treatment for premature ejaculation. However, the interim analyses of phase II data in September 2006 showed that pagoclonone had insufficient efficacy for this indication and development for premature ejaculation was discontinued.

2.3.4 ELB-139

ELB-139 is an imidazolone (Compound 3, [145]) that was evaluated as part of the NIH-sponsored in vivo screening programme to identify novel anticonvulsants [146]. It was being developed by the German company Elbion AG, which was derived from Arzneimittelwerk Dresden GmbH's (AWD) drug discovery business. This compound has low affinity for the $\alpha 1$, $\alpha 2$ and $\alpha 5$ subtypes ($\geq 1.8 \mu\text{M}$) but reportedly has a 30-fold higher affinity for the $\alpha 3$ subtype ($\text{EC}_{50} = 60 \text{ nM}$; Fig. 5e; [124]), which is intriguing given that the structural analogue ELB-138 demonstrates no such $\alpha 3$ selectivity (see below). It has essentially equivalent, partial agonist efficacy at each subtype (relative efficacy values ranging from 0.38 to 0.53). The compound was anxiolytic in the rat elevated plus-maze, light and dark box, and Vogel conflict tests [147] yet showed no signs of sedation. In addition, the compound retained its anxiolytic efficacy on the elevated plus maze following 6 weeks of treatment [147]. Furthermore, there were no signs of tolerance developing to the anticonvulsant effects of ELB-139 in the rat amygdala kindling model [148, 149]. Finally, ELB-139 increased the concentrations of extracellular 5-HT in the striatum and pre-frontal cortex of rats without altering striatal dopamine concentrations, an effect that was blocked by the benzodiazepine site antagonist flumazenil [150].

In healthy male volunteers, ELB-139 was well tolerated either as single doses (ranging from 200 to 1,200 mg) or multiple doses (200–600 mg three times a day) with no signs of overt sedation [149]. EEG recording were used as a pharmacodynamic readout and ELB-139 dose-dependently reduced the α band and enhanced the β band, whilst changes in the δ and θ bands were limited. Effects were observed even at the lowest multiple-dose administration (200 mg; [149]). Elbion were reportedly evaluating the efficacy of ELB-139 following a

35% CO₂ challenge in subjects with a diagnosis of panic disorder in Germany and The Netherlands with a study start date of May 2006 ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00322803), identifier NCT00322803) although results from this study have yet to be disclosed. Following the merger of Elbion AG with 4 AZA in December 2006 to form Elbion NV and the subsequent acquisition of this company by BioTie Therapies in November 2008, the drug was no longer listed as part of the company pipeline. Consequently, development of ELB-139 is presumed to have stopped.

2.3.5 Merck Compounds

Of the new generation of $\alpha 2/\alpha 3$ subtype-selective partial agonists, only clinical data for the triazolopyridazines MRK-409 and TPA023 and the imidazotriazine TPA023B have been published [125–127]. These compounds all have high and equivalent affinity for each of the four GABA_A subtypes with MRK-409 possessing weak partial agonist activity at the $\alpha 1$ subtype whereas TPA023 and TPA023B both lack efficacy at this subtype (Fig. 5f–h). All three compounds produced non-sedating anxiolytic-like profiles in rodent and non-human primate (squirrel monkey) pre-clinical species [125–127, 151].

MRK-409 was progressed into man based upon its pre-clinical anxiolytic profile [125]. However, in single-dose, healthy volunteer studies, the drug surprisingly produced sedation at relatively low doses (1.5 and 2 mg). [¹¹C]Flumazenil PET studies confirmed that this sedation occurred at relatively low levels of GABA_A receptor occupancy (~10%; Fig. 6a). Hence, although this compound (also known as MRK-0343) clearly has a pharmacodynamic profile that distinguishes it from lorazepam [135], these effects were observed at doses of 0.25 and

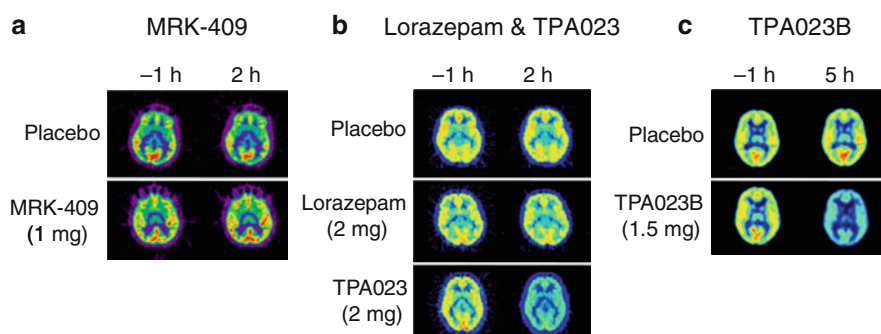


Fig. 6 Representative pseudo-colour positron emission tomography images representing the uptake of [¹¹C]flumazenil in the human brain prior to (–1 h) or either 2 h (MRK-409, lorazepam and TPA023) or 5 h (TPA023B) after oral administration of single doses of either (a) 1 mg MRK-409; (b) 2 mg lorazepam or 2 mg TPA023; or (c) 1.5 mg TPA023B. The times of the first scan after dosing were chosen so they roughly correspond to the T_{\max} for each compound [125, 127, 152]

0.75 mg that are assumed to be too low to be of therapeutic relevance for anxiety disorders [125].

The development of TPA023 for the treatment of GAD was halted due to pre-clinical toxicity (cataract formation) in long-term dosing studies [153]. Nevertheless, combining the data from the three Phase II studies underway at the time development was halted showed that TPA023 gave a significantly greater decrease in the HAM-A score relative to placebo [153]. These clinical studies employed flexible dosing schedules of either 1.5–4.5 mg or 3–8 mg total doses of an extended release (gel extrusion module) formulation, with the main adverse event observed in Phase I studies of the 8-mg dose being dizziness. The corresponding C_{\max} achieved using an 8 mg dose of this formulation (25 ng/ml) corresponds to an occupancy in the region of 70% based upon a single-dose normal volunteer PET study (Fig. 6b; [152]). The lack of overt sedation-like adverse events was confirmed in pharmacodynamic studies, in which TPA023 did not affect parameters such as body sway that are associated with sedation [134]. This compound has been subsequently evaluated in a small population of schizophrenia patients, in which it produced a consistent trend towards improved cognitive function [154]. Like TPA023, the back-up compound TPA023B was also able to achieve significant levels of occupancy in the absence of overt sedation [127, 155]. However, development of this compound was halted for as yet undisclosed reasons.

2.4 Additional Properties Of Non-Sedating Anxiolytics

In terms of developing novel, non-sedating anxiolytics, it is pertinent to discuss how a compound could differentiate itself from generic benzodiazepines. Clearly the lack of, or at the very least a much reduced, sedation liability whilst retaining the anxiolytic efficacy of non-selective compounds would be a minimal requirement. However, an additional key factor would be that of abuse liability. Hence, the lack of abuse potential to support a claim for a drug being non-scheduled would clearly be advantageous. In this regard, no compounds have progressed far enough in clinical development for this issue to be addressed. Nevertheless, in drug discrimination studies, the interoceptive cues associated with $\alpha 2/\alpha 3$ -selective compounds differentiate them from non-selective benzodiazepines [116, 156]. Furthermore, it is encouraging to note that self-injection of TPA023 in baboons does not differ from that of vehicle, even at doses equivalent to complete GABA_A receptor benzodiazepine site occupancy [157]. Moreover, there were no euphoria-like adverse events noted during Phase I studies with this drug [153]. Collectively, these data suggest that subtype-selective compounds may have a lower abuse potential relative to non-selective, full agonist benzodiazepine site modulators [33, 110, 156, 157], although the extent to which this is attributable to a generally lower partial rather than full efficacy profile or is more

related to reduced modulation of a specific GABA_A receptor subtype remains to be clarified.

2.5 Summary of GABA_A Receptors as a Target for Anxiety Disorders

There is a strong pre-clinical rationale to support the hypothesis that selective modulation of $\alpha 2$ and/or $\alpha 3$ -containing GABA_A receptors should be non-sedating anxiolytics. Moreover, it would appear that even relatively modest efficacy at the $\alpha 1$ subtype translates into sedation in man, based upon observations with bretazenil [120], MRK-409 [125] and EVT-201 (see below). From a medicinal chemistry point of view, $\alpha 2/\alpha 3$ -preferring compounds can be identified using a selective efficacy rather than a selective affinity approach [104, 117] and although the development of the Merck compounds TPA023 and TPA023B had to be halted due to pre-clinical toxicity issues, other companies are continuing the search for anxiolytic compounds acting via the benzodiazepine binding site, although the structures and in vitro and pre-clinical in vivo profiles of these compounds have yet to be disclosed.

NSD-721 is a GABA_A subtype-selective modulator being developed by NeuroSearch in collaboration with GSK for the treatment of social anxiety disorder, with Phase I studies commencing in August 2009. AstraZeneca are evaluating AZD7325 in two proof-of-concept Phase II studies in GAD (ClinicalTrials.gov identifiers NCT00808249 and NCT00807937), both of which have been completed although no data has yet been disclosed. However, a separate study to assess the absorption, distribution, metabolism and excretion of AZD7325 after intravenous and oral administration (ClinicalTrials.gov identifier NCT00940641) has been suspended with the “study withdrawn prior to enrolment due to AZ business decision unrelated to safety” (clinicaltrials.gov). In addition, AstraZeneca have completed Phase I studies in healthy male volunteers using a further compound, AZD6280. These include a comparison of the pharmacodynamic effects (sedation, cognition and EEG) of AZD6280 and lorazepam (NCT00750802) as well as a [¹¹C]flumazenil PET study to measure the occupancy of human brain GABA_A receptor by AZD6280 (NCT00681746).

3 GABA_A Receptor Compounds for Sleep Disorders

3.1 Insomnia

It has been estimated that around 10–15% of adults suffer from chronic insomnia with an additional 25–35% having occasional difficulties sleeping [158]. In addition,

up to around 40% of individuals with insomnia also having a co-existing psychiatric disorder [159]. The primary goals for insomnia treatments should be an improvement in sleep quality and/or total sleep time along with a reduction in sleep latency and wakefulness after sleep onset. In addition, a decrease in insomnia-related daytime impairments such as attention difficulties, cognitive dysfunction and fatigue are also desirable [160, 161]. The guidelines for the development of hypnotic drugs tend to focus on primary insomnia and use sleep-related metrics. However, sleep disturbances are a common feature of the symptomatology of a variety of additional disorders, such as depression, anxiety and Alzheimer's disease. Accordingly, daytime functional readouts (e.g., depression, anxiety or cognition rating scale) may be more useful parameter for examining efficacy in these disorders in which disrupted sleep is a co-morbidity [162].

A number of treatment options are available for insomnia, including psychological and behavioural therapies as well as pharmacological approaches [163]. With regard to the latter, FDA-approved treatments include several GABA_A receptor benzodiazepine site modulators (Table 1) as well as the melatonin receptor antagonist ramelteon. Of the benzodiazepine site modulators, the short/intermediate half-life benzodiazepines temazepam and triazolam as well as the longer-lasting and less popular estazolam, flurazepam and quazepam are all approved for the treatment of insomnia. However, as regards flurazepam, Hollister commented that "I have always thought that flurazepam, a drug that requires biotransformation to become active, and the metabolite of which has a very long life-span, was badly miscast as an hypnotic" [31]. Other benzodiazepines not approved for insomnia, such as lorazepam or clonazepam, might also be used as hypnotics [161]. In addition to

Table 1 Summary of the principle pharmacokinetic parameters of FDA-approved and experimental GABA_A-mediated treatments for insomnia

Drug	Trade name	Status	Structure	Pharmacokinetic parameters ^a	
				<i>T</i> _{max} (h)	<i>T</i> _{1/2} (h)
Estazolam	ProSom	FDA approved	Benzodiazepine	2	8–24
Flurazepam	Dalmane	FDA approved	Benzodiazepine	2	48–96 ^b
Quazepam	Doral	FDA approved	Benzodiazepine	2	15–40
Temazepam	Restoril	FDA approved	Benzodiazepine	0.8	11
Triazolam	Halcion	FDA approved	Benzodiazepine	1.3	2.9
Zolpidem IR	Ambien	FDA approved	Imidazopyridine	1.0	1.0–2.6
Zaleplon	Sonata	FDA approved	Pyrazolopyrimidine	1.1	1.0
Zopiclone	Imovane	Approved outside US	Cycloprrrolone	1.0	5.6
Eszopiclone	Lunesta	FDA approved	Cycloprrrolone	1.6	6.5
Adipiplon	N/A	Experimental	Triazolopyrimidine	1	1.1
Indiplon	N/A	Experimental	Pyrazolopyrimidine	1	2–4
EVT-201	N/A	Experimental	Unknown	1–2	3–4
THIP (Gaboxadol)	N/A	Experimental	GABA site agonist	0.5–2	1–2

In addition, the melatonin receptor agonist ramelteon is also approved for the treatment of insomnia

^aPharmacokinetic data are derived from: [164–174]

^bThe half-life value for flurazepam refers to the active metabolite *N*1-desalkyl-flurazepam

non-selective benzodiazepines, the so-called “Z-drugs” are also FDA-approved hypnotics. These drugs include zolpidem (Ambien), zaleplon (Sonata) and (S)-zopiclone (eszopiclone; Lunesta), the latter of which is the active enantiomer of racemic zopiclone (Imovane), which is approved for use as a hypnotic outside the US. Although a variety of benzodiazepines have been evaluated as hypnotics [164, 175], the preferential use of the benzodiazepines temazepam and triazolam in the treatment of insomnia is based upon the relatively short half-life of these compounds rather than any differences in pharmacology relative to other non-selective benzodiazepines [161].

The Z-drugs (zolpidem, zaleplon, zopiclone and eszopiclone) are all non-benzodiazepine chemical structures but exert their actions by modulating GABA_A receptor function via the benzodiazepine binding site. These compounds are all characterised by relatively short halves in man (~1–6 h, Table 1; [176]), and in the case of zolpidem, a higher affinity for GABA_A receptors containing an $\alpha 1$ rather than $\alpha 2$, $\alpha 3$ or $\alpha 5$ subunit, which is of particular relevance to the pharmacology of zolpidem as this subtype is associated with the sedating properties of benzodiazepines [40, 42]. However, although $\alpha 1$ -preferring compounds are more selectively hypnotic compared to non-selective benzodiazepines, there are no conclusive data to suggest that such compounds have improved efficacy or reduced side effects relative to non-selective benzodiazepines [177–180].

Benzodiazepine and Z-drug hypnotics have the same dependence and abuse potential risks associated with the use of benzodiazepines in the treatment of anxiety disorders (see above). In addition, post-marketing surveillance has recently identified a number of relatively rare, and therefore poorly understood, sleep-associated behaviours related to the use of hypnotics, such as preparing and eating food, making phone calls and sleep-driving [181]. A further issue associated with the pharmacological effects of hypnotics acting via the benzodiazepine recognition site is that of residual daytime sedation (“hangover”), a side effect most noticeable with longer half-life benzodiazepines such as flurazepam or nitrazepam [182]. More specifically, the “hangover” effect relates to residual daytime sleepiness and impairment of cognitive and/or psychomotor functioning the day after drug administration prior to bedtime on the previous day [164]. As a consequence of these effects, patients may have an increased risk of falls and hip fractures, which is of particular concern in the elderly [114], or of traffic accidents. An epidemiological study showed that the risk of an accident increased as the half-life of the hypnotic increased, although even short half-life drugs were also associated with an increased risk [164]. Clearly, in order to minimise the degree of next-day effects, it is desirable to use drugs with a short half-life [182]. In addition, it could be argued that because relatively weak efficacy at the $\alpha 1$ subtype is sufficient to produce sedation in man [125], full agonism at this subtype is not only more than sufficient to produce sedation but might also actually contribute to the “hangover” effects associated with such compounds. In this regard, a partial agonist hypnotic, such as EVT-201 (see below), might be of particular interest since it could well be devoid of some of the side effects associated with the full agonist hypnotics.

3.2 Marketed Non-Benzodiazepine GABA_A-Modulating Hypnotics: The “Z-Drugs”

In the present section, the properties of zolpidem, zaleplon, zopiclone and (*S*)-zopiclone are briefly discussed, particularly with respect to their *in vitro* GABA_A receptor subtype selectivity profiles since this forms the basis for the comparisons with the newer drugs described in the subsequent section. However, more detailed reviews of these drugs are available elsewhere [165, 166, 183–188, 275–277].

Zolpidem: The imidazopyridine zolpidem (Ambien) is the prototypic $\alpha 1$ -preferring sedative hypnotic [189, 190]. It has around 5–14-fold higher affinity for the $\alpha 1$ compared to $\alpha 2$ and $\alpha 3$ subtypes (Fig. 7a). Interestingly, it has very low affinity for the $\alpha 5$ subtype but this is not thought to play a major role in differentiating zolpidem from the non-selective benzodiazepines. Zolpidem is rapidly absorbed with a T_{\max} of around 1 h and is cleared with a plasma half-life of 2.5 h or less (Table 1). It reduces the latency to sleep onset and the number of night-time awakenings as well as increasing the duration of sleep [191]. More recently, an extended-release formulation of (zolpidem CR) has been approved and is intended for patients with poor sleep maintenance [194]. This modified release formulation is a bilayer tablet comprising an immediate release portion, designed to induce sleep, as well as a slower release formulation intended to maintain sleep [195–197]. In addition to a bilayer tablet, zolpidem has also been reformulated in a number of other ways, including sublingual and spray formulations [198]. In human sedative drug abusers, neither zolpidem nor zaleplon differed from triazolam with respect to subjective ratings of drug liking [199].

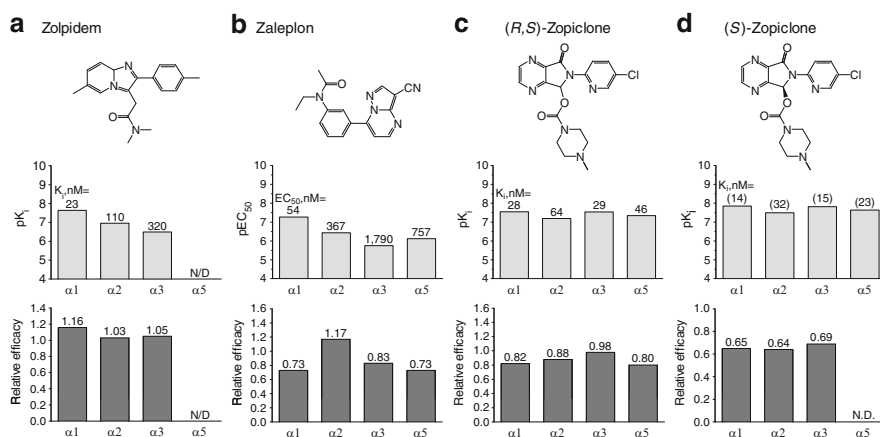


Fig. 7 Structure and *in vitro* and *in vivo* efficacy profiles of non-benzodiazepine, approved sedative-hypnotics. Data are taken from: zolpidem, [24]; zaleplon, [191]; (*R,S*)-zopiclone, [24]. For (*S*)-zopiclone (eszopiclone), the affinity was calculated based upon the affinity of the racemate (panel C) assuming that the (*S*)-enantiomer has an affinity approximately 2-fold higher than the racemate [192] whereas the relative efficacy was calculated by multiplying the relative efficacy values of the racemate (panel C) by the ratio of the increase in GABA currents produced by the (*S*)-enantiomer and the racemate [193]. *N.D.* not determined

Zaleplon: Zaleplon is a pyrazolopyrimidine, which, like zolpidem, has modestly higher affinity for the $\alpha 1$ compared to $\alpha 2$ and $\alpha 3$ subtypes (Fig. 7b), although the affinity at the $\alpha 1$ subtype is about twofold lower than zolpidem. Unlike zolpidem, zaleplon also has affinity for the $\alpha 5$ subtype [191, 278]. Interestingly, zaleplon has 10–20-fold higher affinity for $\gamma 3$ compared to $\gamma 2$ subunit-containing GABA_A receptors [200], whereas zopiclone has similar affinity for the $\gamma 2$ and $\gamma 3$ subtypes and zolpidem has essentially no affinity for the $\gamma 3$ subtypes. However, the pharmacological significance of the higher $\gamma 3$ compared to $\gamma 2$ affinity of zaleplon remains uncertain. It has a short T_{\max} and elimination half-life, both of which are in the region of 1 h (Table 1).

Zopiclone and Eszopiclone: Zopiclone (Imovane) is a cyclopyrrolone which, although not approved for use within the US, has been available in Europe for many years. It has no selectivity for the different α subunit-containing GABA_A subtype (Fig. 7c). Zopiclone is a racemate comprising *R*- and *S*-enantiomers with the latter possessing around 50-fold higher affinity for GABA_A receptors than the former and the *S*-enantiomer being around twofold higher affinity than the racemate [192]. Accordingly, in animals, (*S*)-zopiclone (eszopiclone) produces sedation to a similar extent as (*R,S*)-zopiclone whereas (*R*)-zopiclone is much less potent [201]. Compared to racemic zopiclone, eszopiclone appears to have a reduction in next-day effects [176, 202] despite the fact that with a half-life in the region of 6 h (Table 1), significant amounts of drug would be predicted to be present at the beginning of the next day.

3.3 Non-Benzodiazepine GABA_A-Modulating Hypnotics in Clinical Development

3.3.1 Adiplon

Adiplon (NG2-73) is a triazolopyrimidine that was being developed as a hypnotic by Neurogen (recently acquired by Ligand Pharmaceuticals). Although claimed to be an $\alpha 3$ -preferring compound [203], when efficacy values are normalised relative to a non-selective benzodiazepine full agonist, the compound has more of a non-selective partial agonist profile (Fig. 8a).

Based on rodent data, the minimally efficacious plasma concentration was predicted to be 2.6 ng/ml which, when corrected for plasma protein binding, gave a target human plasma concentration of 3.4 ng/ml [206]. In a transient insomnia model in healthy volunteers, a 1-mg dose of adiplon produced a significant decrease in the latency to persistent sleep, with a C_{\max} in the region of 2.1 ng/ml and a T_{\max} of ~ 1 h and a half-life of 1.1 h [167].

The compound was formulated into a bilayer tablet containing immediate- and controlled-release formulations, and the 6- and 9-mg doses were evaluated for efficacy in insomnia relative to zolpidem CR in a 60 patient double-blind Phase II/III study that commenced in July 2008. However, unwanted next-day effects were observed in a dose-dependent manner, and the trial was suspended pending

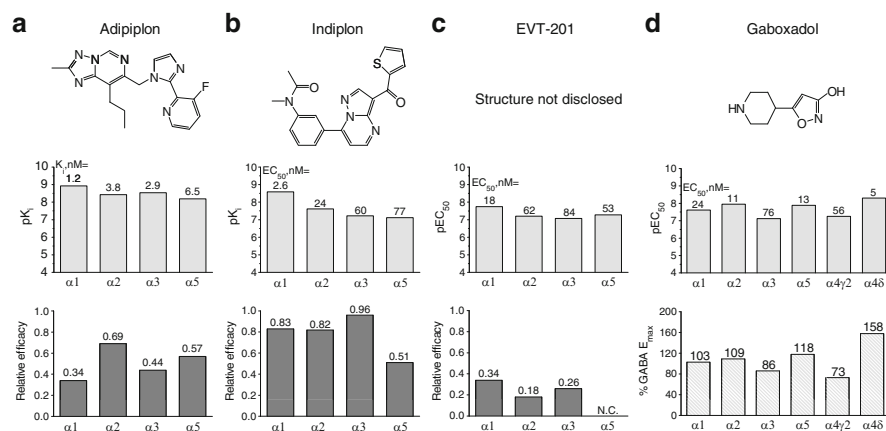


Fig. 8 Structure and in vitro binding and relative efficacy profiles of GABA_A receptor-mediated sedative-hypnotics that exert their effects either via the benzodiazepine binding site (adiplon, indiplon or EVT-201) or the GABA recognition site (gaboxadol). Data are taken from: adiplon [203]; indiplon, affinity [204], efficacy [191]; EVT-201 [205]; Gaboxadol [191]. As regards EVT-201, the efficacy was calculated by multiplying the relative efficacy values of zolpidem (Fig. 7a) by the ratio of the increase in GABA currents produced by the EVT-201 and zolpidem [205]. N.C. not calculated

further studies of the bilayer tablet formulation. However, in November 2008, Neurogen announced that further studies with adiplon were not planned. The Neurogen group have also described NDT 9530021 as an additional $\alpha3$ -preferring compound that is structurally related to adiplon [207, 208]. This compound has an efficacy profile very similar to that of adiplon [207] and was a potent hypnotic, anxiolytic and anticonvulsant but, in rats, there was little separation between the anxiolytic, anticonvulsant and motor-impairing doses [207, 208].

3.3.2 Indiplon

Indiplon (also known as NBI 34060) is a pyrazolopyrimidine (Fig. 8b) that was acquired by Neurocrine Biosciences in 1998 from DOV Pharmaceuticals, who themselves had earlier licensed the compound from American Cyanamid Co. (now Wyeth). Indiplon has a degree of selectivity for the $\alpha1$ subtype of GABA_A receptors [209] that varies from 10–30-fold [204] to a more modest 2–4-fold [191]. The compound has equivalent, full agonist efficacy at each of the $\alpha1$, $\alpha2$, $\alpha3$ and $\alpha5$ subtypes and accordingly has marked sedative effects in rodents [210]. Indiplon has a short half-life in rodents (1 h) [210] as well as in man (2–4 h) [168, 211] and in human volunteers with a history of drug abuse, the abuse potential of indiplon did not differ from that of the non-selective benzodiazepine triazolam [212].

Indiplon was developed as both an immediate-release capsule and a modified-release tablet (indiplon-IR and indiplon-MR, respectively) with the latter being

designed to provide an initial dose of drug followed thereafter by a gradual release over an extended period of time. Indiplon-IR has been evaluated in ten studies, of which four were in the elderly whereas indiplon-MR was assessed in four studies, two of which involved the elderly [168, 211]. Hence, most clinical data is derived from the double-blind, placebo-controlled studies using the IR formulation (a total of 3,105 healthy volunteers and insomnia patients between the ages of 18–80) rather than the indiplon-MR formulation (536 subjects aged between 19 and 85) [168, 211]. As regards the MR formulation, in a two-week study in elderly subjects with insomnia, 15 mg indiplon-MR significantly decreased the self-reported latency to sleep onset and the time spent awake after sleep onset whilst increasing the rating of sleep quality and total sleep time [213]. However, the 15 mg MR tablet received a Not Approvable letter in May 2006 with the FDA requesting a long-term safety and efficacy trial in the elderly and it would appear that this formulation is no longer being pursued.

In May 2006, the US Food and Drug Administration (FDA) issued an Approvable letter for the 5 and 10 mg indiplon-IR capsules and requested a reanalysis of some data to support the indications of sleep initiation and middle of the night dosing [168]. In June 2007, a New Drug Application was submitted to the FDA and an Approvable Letter was issued for the 5 and 10 mg doses in December 2007 pending the outcome of a study in the elderly, a study comparing the adverse events of indiplon versus an approved insomnia treatment and finally, a pre-clinical study of indiplon to support its use in the third trimester of pregnancy [211]. At this time, indiplon-IR remains unapproved, and even if approved, it is uncertain if it will have a specific advantage or indication compared to other insomnia medications. Furthermore, although the use of indiplon-IR for post-bedtime or middle of the night dosing may represent a potential advantage [214], zaleplon is already used off-label in a similar manner [186].

3.3.3 EVT-201

EVT-201 was licenced from Roche to Evotec and was originally developed by Roche as a non-sedating anxiolytic based upon pre-clinical data yet the compound caused sedation in human Phase I studies. Although the structure of EVT-201 has not been publically disclosed, it is a partial agonist [205] with a slight, 2–4-fold higher functional affinity at the $\alpha 1$ compared to $\alpha 2$, $\alpha 3$ and $\alpha 5$ GABA_A receptor subtypes (Fig. 8c). It has an active metabolite, desmethyl-EVT-201, which has a functional affinity very similar to the parent but with an efficacy that is around half of that of EVT-201, therefore making it a weak partial agonist [205]. In pre-clinical models, EVT-201 clearly differentiates from non-selective agonists with anxiolytic-like activity in pre-clinical species being observed at doses associated with no motor impairment, whereas in comparison there was an overlap of the anxiolytic and motor-impairing doses of alprazolam [169].

In a Phase II study in adults with primary insomnia, 1.5 and 2.5 mg doses of EVT-201 significantly decreased the wake after sleep onset and increased total sleep time and there was no subjective residual sedation [169]. A second study in elderly

subjects with primary insomnia and daytime sleepiness also demonstrated an increase in the total sleep time [215]. Most notably, EVT-201 differentiates itself from the existing FDA-approved non-selective benzodiazepines as well as the Z-drugs by virtue of having much lower efficacy at each of the four GABA_A receptor subtypes. In addition, since the metabolite has lower efficacy than the parent, this may also further attenuate the effects of EVT-201. Despite this, EVT-201 retains clinical efficacy in adult and elderly patients with primary insomnia [169, 215] and is consistent with other observations that full agonist efficacy at the $\alpha 1$ subtype is not required to produce sedation in man [120, 125]. Furthermore, irrespective of which subtypes are associated with the next-day hangover effects produced by hypnotics that act via the benzodiazepine binding site, it is likely that a partial agonist, such as EVT-201, will have a lower propensity to cause such side effects. Currently, Evotec are seeking a partner for the further development of EVT 201.

3.3.4 Gaboxadol

Gaboxadol (THIP) was originally described in 1977 [20] and is a conformationally restricted analogue of muscimol (Fig. 2a). It readily crosses the blood-brain barrier and was first developed as a potential treatment of acute pain, anxiety, epilepsy, schizophrenia and/or Huntington's disease [216]. Although initially described as a partial agonist, gaboxadol actually has higher efficacy than GABA at the $\alpha 4\beta\delta$ subtype (Fig. 8d; [216–218]), which is of particular interest as this subtype of GABA_A receptors is generally thought to be localised extrasynaptically [219–221]. Neuroanatomically, the *in vivo* effects of gaboxadol may in part be mediated via the neurons of the ventrobasal thalamic nucleus [65, 222]. Furthermore, based on rat data, it would appear that despite the relatively modest $\alpha 4\beta\delta$ binding and efficacy selectivity of gaboxadol (Fig. 8d), plasma drug concentrations at a hypnotic dose are consistent with a selective activation of this subtype compared to $\alpha 1$, $\alpha 2$ -, $\alpha 3$ -, $\alpha 4$ - or $\alpha 5\beta 3\gamma 2$ receptors [179], although clearly such calculations encompass a multitude of assumptions and extrapolations that may or may not be applicable to man.

Gaboxadol is rapidly absorbed in man, achieving peak plasma concentrations between 0.5 and 2 h and has a short, 1.5–2 h, half-life [170, 171]. As regards sleep architecture, in man, as in rats, gaboxadol produces a significant increase in slow-wave sleep without suppressing REM [223–225], and the changes in the EEG sleep spectrum produced are markedly different from those associated with compounds acting via the benzodiazepine binding site [191]. The difference in the mechanism of action between gaboxadol and benzodiazepine site modulators is further emphasised by the failure of gaboxadol to produce the same interoceptive cues as either the non-selective benzodiazepines, the marketed hypnotics such as zolpidem, zopiclone, zaleplon, or the experimental hypnotic indiplon [226, 227].

In healthy elderly subjects, gaboxadol significantly decreased the subjective sleep-onset latency as well as increasing sleep intensity and quality as measured by self-assessment [225]. Moreover, there were no signs of next-day effects in this subject population [228]. In a phase-advance model of transient insomnia, gaboxadol decreased

the latency to persistent sleep as well as the wakefulness after sleep onset and increased the total sleep time [229, 230]. In an additional experimental medicine model, namely an impairment in night time sleep produced by a late afternoon nap in healthy young volunteers, gaboxadol increased total sleep time and the amount of slow wave sleep as well as enhancing the subjective sleep quality relative to placebo treated subjects [231].

In primary insomnia, two initial small-scale exploratory, polysomnographic studies in adults aged 18–65 years ($n = 23$ and $n = 38$; [232, 233], respectively) demonstrated efficacy of gaboxadol following short-term (2-day) treatment with either 5 or 15 mg [232] or 10 and 20 mg doses [233]. These observations were extended to a large 2-week Phase III study of 5, 10 and 15 mg doses of gaboxadol in 18–65 year old adults with insomnia ($n = 742$, $n = \sim 140$ –150/treatment group) with outcome measures (subjective self-assessments recorded using electronic diaries) being compared to placebo and 10 mg zolpidem [234]. Generally speaking, the 15 mg dose of gaboxadol significantly improved a variety of sleep onset and maintenance parameters over the 2-week period; an effect that was comparable to, or slightly less than that observed with zolpidem [234]. Furthermore, although zolpidem shows signs of rebound insomnia following cessation of treatment, gaboxadol did not [234].

In two additional large Phase III studies of efficacy in primary insomnia, wake after sleep onset and latency to persistent sleep were used as the primary end-points in separate 30-night polysomnographic studies to assess the efficacy of gaboxadol in either adults (18–64 years old) given placebo or 10 or 15 mg gaboxadol ($n = \sim 150$ /group) or elderly subjects (≥ 65 years old) receiving either placebo or 5 or 10 mg gaboxadol ($n = \sim 150$ –175/group; [235]). Comparisons were made between parameters averaged over nights 1 and 2 and over nights 29 and 30. Although in both studies the higher dose of gaboxadol was effective at decreasing the wake after sleep onset, it had no effect on the latency to persistent sleep in the adult study, and although the higher dose improved the latency to persistent sleep in the elderly study on Nights 1/2, this was not maintained through to Nights 29/30 [235]. Based upon these equivocal Phase III data and observations of psychiatric side effects at supratherapeutic doses in an abuse liability study involving drug abusers [235], Lundbeck and Merck announced the discontinuation of their joint gaboxadol development programme in March 2007. However, a characteristic effect of gaboxadol is its pronounced effect on slow-wave sleep; yet this drug was being developed in for an indication of primary insomnia, a condition where disrupted slow-wave sleep is not a main feature [236]. Accordingly, the effects of gaboxadol on subjects with impairments in slow-wave sleep remain unknown [162].

3.4 Summary of GABA_A Receptors as a Target for Hypnotics

The GABA_A receptor is clearly a well validated target for hypnotics based on clinical experience with the non-selective full agonist benzodiazepines as well as the non-selective and $\alpha 1$ -selective non-benzodiazepine “Z-drugs”. Of the next-generation of

GABA_A-mediated hypnotics, development of the benzodiazepine site modulators adiplon and indiplon as well as the $\alpha 4\beta\delta$ -preferring GABA-site agonist gaboxadol has halted, for a variety of reasons, leaving just EVT-201 in clinical development. EVT-201 differs from other marketed and experimental benzodiazepine site-mediated hypnotics in that it has relatively weak, partial agonist efficacy and only a modest 2–4-fold higher affinity for the $\alpha 1$ compared to $\alpha 2$, $\alpha 3$ and $\alpha 5$ subtypes. Nevertheless, the modest $\alpha 1$ efficacy is sufficient to demonstrate efficacy in insomnia patients [169, 215] and the low efficacy at the $\alpha 2$, $\alpha 3$ and $\alpha 5$ subtypes may well be associated with a reduction in side effects (“hangover”).

4 Epilepsy

Epilepsy is defined as the occurrence of two or more seizures not provoked by any identifiable cause. It is not a single disease but rather reflects a variety of disorders arising from many different types of brain dysfunction [237]. Seizures may be either partial (focal), in which the seizure begins in a local area of the brain, or generalised, which involves the whole brain simultaneously. Collectively, the various forms of epilepsy have a lifetime incidence in the region of 15–50/100,000/year and a prevalence of active epilepsy of 3–18/1,000 [238, 239]. Status epilepticus is defined as a single seizure that lasts for over 30 min without a recovery of consciousness, although particularly for tonic–clonic seizures, a period of 5 min should be viewed as status epilepticus and such a seizure may or may not be associated with epilepsy [240, 241]. When not associated with an underlying epilepsy disorder a status epilepticus seizure may occur as a consequence of, for example, an acute illness or insult or as the result of intoxication or drug abuse. Status epilepticus has an average incidence of around 20/100,000/year in the industrialised world and short-term mortality rate in the region of 20% [241, 242]. It has a bimodal age distribution, with the highest incidences being during the first year of life, generally as a result of febrile seizures, and after the age of 60 when cerebrovascular diseases, including acute stroke and haemorrhage, are major risk factors [240, 241]. It has been estimated that 12–30% of adults with a new diagnosis of epilepsy first present with status epilepticus [82].

The goals for the treatment of epilepsy should be complete seizure control, thereby permitting a normal lifestyle, with minimal or preferably no side effects [243–245]. However, up to one-third of epilepsy patients continue to experience seizures or unacceptable side effects and are therefore refractory as defined by the failure of two appropriately chosen and used antiepileptic drugs to prevent seizures [246]. As GABA is the major inhibitory neurotransmitter in the CNS, the strategy of inhibition of the intracellular degrading enzyme GABA transaminase with vigabatrin, thereby increasing intracellular and secondarily extracellular GABA levels, or inhibition of the plasma membrane GABA transporter GAT-1 by tiagabine represent rationale approaches to the development of novel antiepileptic drugs. However, these strategies are relatively non-selective in so far as effects will be

mediated via both the ionotropic GABA_A and metabotropic GABA_B families of receptor [247]. An alternative and more selective approach for increasing GABA-mediated inhibition would be to enhance GABA_A receptor function, especially given that mutations in genes that comprise this receptor family have been associated with idiopathic generalised epilepsies and thereby underscore the importance of these receptors in seizure activity [247–249]. Pharmacologically, the clinical use of barbiturates, particularly phenobarbital [83], and benzodiazepines (see below) in the treatment of epilepsy and status epilepticus further highlight the GABA_A receptor as a pharmacological target for the development of novel antiepileptic drugs. The goal of such drugs should be to maintain efficacy yet at the same time reduce the various liabilities associated with modulating GABA_A receptor function via either barbiturate, benzodiazepine or other recognition site.

4.1 Use of Benzodiazepines in the Treatment of Epilepsy

The anticonvulsant effects of benzodiazepines were reported shortly after their introduction [250], and although a number of new antiepileptic drugs have been launched during the past 15 years, benzodiazepines remain the first-line treatment for status epilepticus and seizures occurring following an anoxic insult [82, 251]. The most common benzodiazepines used in the treatment of status epilepticus are diazepam, lorazepam and midazolam, primarily due to the variety of formulations and dosing routes available for these particular drugs [251]. A comparison of the effectiveness of lorazepam and diazepam in the treatment of status epilepticus tends to favour lorazepam [252–254]. However, differences between benzodiazepines are more related to variations in pharmacokinetic rather than pharmacological properties [251], with lorazepam possibly being preferred as a consequence of its slower rate of distribution within the body [240]. Given the efficacy of non-selective, full agonist benzodiazepines in the treatment of status epilepticus plus their associated sedative and cognition impairing effects, which for the treatment of status epilepticus are actually very desirable features, there would appear to be little potential benefit of subtype-selective GABA_A modulators for this indication.

Despite being a first-line choice for the treatment of status epilepticus, benzodiazepines are unsuitable for the prophylactic treatment of epilepsy due to the development of tolerance, which is defined as the decrease in the pharmacological effects of a drug over a period of time. In terms of antiepileptic drugs, tolerance is associated with an increase in the number and severity of seizures as well as an increased risk of seizures following cessation of treatment. Tolerance to the anticonvulsant effects of benzodiazepines has been demonstrated pre-clinically, and while this may be overcome to a certain extent by increasing the dose, benzodiazepines are, nevertheless, considered unsuitable for the long-term control of epilepsy [255, 256]. In pre-clinical species, the rate at which tolerance develops differs between benzodiazepines [257, 258], and while the mechanisms of tolerance are poorly understood [259, 260], they are thought to involve an uncoupling between

the GABA and benzodiazepine binding sites [261]. Hence, the challenge is to develop a GABA_A receptor modulator that is devoid of the tolerance observed with the non-selective benzodiazepines and which is therefore appropriate for prophylactic use.

4.2 Novel Pre-clinical Benzodiazepine Site Modulator: ELB-138

ELB-138 (Compound 2, [145]) is an imidazolone that was originally evaluated as part of the NINDS Antiepileptic Drug Development Programme [146] and proved to have anticonvulsant activity in a number of mouse, rat and dog seizure models at doses below those associated with motor impairment. It has low affinity for GABA_A receptors (K_i in rat cortex of 4.4 μ M [145]) and at the different subtypes has efficacy relative to diazepam ranging from 0.27 to 0.43, with the EC_{50} of between 2.4 and 9.4 μ M [262] (Fig. 9). However, it should be noted that these efficacy values may be an underestimate of the true relative efficacy since no apparent maximal effects were observed at a concentration of 10 μ M and therefore the relative efficacy and EC_{50} values may well be higher than those reported [262].

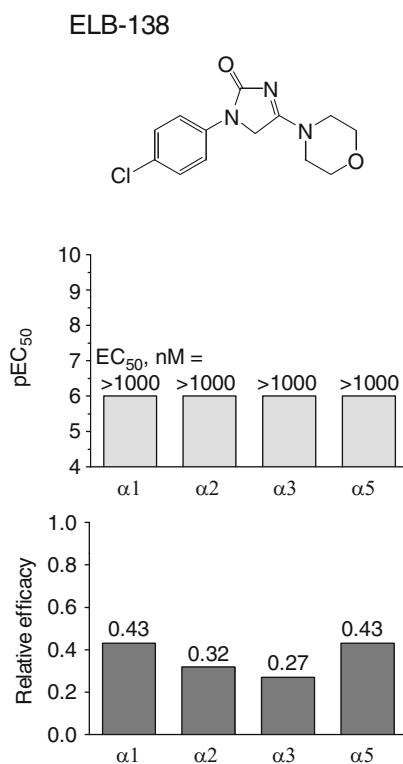


Fig. 9 Structure and in vitro binding and relative efficacy profile of ELB-138. Since the EC_{50} could not be accurately determined from the concentration-effect curves generated in *Xenopus laevis* oocytes, the EC_{50} at each subtype is stated as being >1,000 nM. Data are from [262]

This compound did not produce midazolam-like responding in a squirrel monkey drug-discrimination assay, and in the same species the rate of self-injection declined to vehicle levels when ELB-138 was substituted for cocaine in a self-administration assay, data which collectively suggest that ELB-138 has a low abuse liability [263].

ELB-138 was claimed to be in Phase I clinical development, but its fate in man is unclear. Based upon the observations that in dogs there is no tolerance to the anticonvulsant effects of ELB-138 [264], a pilot clinical study was performed in dogs with epilepsy. This study showed that ELB-138 was efficacious and had a reduced side-effect profile relative to conventional medications with the median plasma levels achieved in this study being in the region of 4,000–7,500 ng/ml 2 h after dosing of 10–15 mg/kg [265]. These plasma concentrations are in the regions of the concentrations (~5,000 ng/ml) required to produce around 20% occupancy of rat brain GABA_A receptors (JA, unpublished observations).

4.3 Neurosteroids: Ganaxolone

Ganaxolone (CCD 1042, 3 α -hydroxy-3 β -methyl-5 α -pregnan-20-one; Fig. 2c) is the 3 β -methylated synthetic analogue of the progesterone analogue allopregnanolone (3 α -hydroxy-5 α -pregnan-20-one; [71]). It was originally developed as an anticonvulsant therapy by CoCensys (which was subsequently acquired by Purdue Pharma), but development was suspended in the late 1990s [266]. This compound has demonstrated anticonvulsant efficacy in a number of pre-clinical models [71, 266, 267] and, importantly, does not appear to demonstrate tolerance after dosing for 7 days [268]. There is evidence that neurosteroids may selectively modulate extrasynaptic, δ subunit-containing GABA_A receptors [65], although the extent to which the anticonvulsant effects of ganaxolone can be attributed to this subtype is unclear. In a preliminary, placebo-controlled clinical trial in treatment-refractory patients with complex partial seizures and that were withdrawn from antiepileptic drugs during pre-surgical evaluation (placebo and ganaxolone group sizes = 28 and 24, respectively), ganaxolone (1,500 mg/day on Day 1 and 1,875 mg/day on Days 2–8) was shown to have antiepileptic-like activity [269]. Three Phase II, open-label, adjunctive studies have also been conducted in a total of 79 paediatric subjects (ranging from 6 months to 15 years old) with refractory seizures using 2–9 week titration followed by an 8 week maintenance period at doses of 12 mg/kg three times a day [270, 271]. There was an improvement in seizure frequency and behavioural improvement, which resulted in 29 subjects continuing ganaxolone treatment [272].

In September 2004, the rights to ganaxolone were acquired by Marinus Pharmaceuticals who are now actively developing this compound [273]. In early 2009, the company announced that ganaxolone (1–2 week escalation to an 8-week maintenance dose of 1,500 mg/day) was safe, well tolerated and efficacious as an adjunctive therapy in adults with partial onset seizures using a reduction in mean weekly

seizure frequency versus placebo as the primary endpoint. Moreover, efficacy was observed within the first week of dosing (www.marinuspharma.com/nr_positive_results.html). However, a common adverse event in clinical studies with ganaxolone is somnolence [273], which in healthy volunteers appears to occur at plasma concentrations greater than 300 ng/ml [271].

When complexed with β -cyclodextrin, ganaxolone showed a marked food-effect following oral dosing in man. More specifically, exposure was 5–15-fold higher when administered in the fed state compared to the fasted state [271], and consequently, Marinus Pharmaceuticals are currently investigating different non-cyclodextrin based formulations of ganaxolone in order to address the issue of variable absorption [245, 271], and a clinical study in infants and young children with infantile spasms is ongoing [271]. In addition, and based upon pre-clinical data, ganaxolone might be relevant to the treatment of catamenial epilepsy, a condition whereby women with epilepsy often report an increased incidence of seizures at the time of menstruation [266, 274].

4.4 Summary of GABA_A Receptors as a Target for Epilepsy

Clearly, the GABA_A receptor is a suitable target for novel anti-epileptic drugs with the challenges being the maintenance of the efficacy produced by the non-selective full agonist benzodiazepines while at the same time eliminating the tolerance associated with such drugs. The desirability of sedative properties is dependent upon the clinical use of the drug with sedation being advantageous for the treatment of status epilepticus whereas it is undesirable for prophylactic use as an antiepileptic drug. As regards modulation of the GABA_A receptor via the benzodiazepine site, there does not appear to be a particular subtype associated with tolerance and it is unlikely that a compound targeting a single subtype would possess the anticonvulsant efficacy of a non-selective full agonist [44]. However, the variety of other recognition sites on the GABA_A receptor provide alternative, non-benzodiazepine site-mediated opportunities for modulating receptor function as does the distinction between synaptic and extrasynaptic localisation; yet these avenues remain relatively unexplored in the context of anticonvulsant activities.

5 Overall Summary

The clinical use of drugs that alter GABA_A receptor function, including barbiturates and, most notably, benzodiazepines as anxiolytics, hypnotics and anticonvulsants provides ample proof of concept that the GABA_A receptor represents a validated target for the development of next-generation treatments for GAD, insomnia and epilepsy. Moreover, the multiple GABA_A receptor subtypes that mediate these clinical effects suggests that targeting specific subtypes of this receptor population

may well lead to novel drugs with an improved clinical profile. Clearly, the desired profile varies according to the indication. Hence, a non-sedating anxiolytic is needed for the treatment of GAD, a drug with rapid onset and no next-day hangover is required for insomnia and other sleep disorders, and for epilepsy, a drug that does not develop tolerance, thereby permitting its use as a prophylactic, is desired. Some of these aspects are more related to pharmacokinetics rather than pharmacology (for example, the speed of onset for a hypnotic). However, as regards pharmacology, there is a strong pre-clinical rationale for the development of anxioreselective compounds based upon selective activation of the $\alpha 2/\alpha 3$ subtypes. With respect to epilepsy, although the molecular mechanisms underlying the tolerance to the anticonvulsant effects of benzodiazepines remain poorly understood and represent a major obstacle to next generation antiepileptic drugs that act via this binding site, there are a variety of different binding sites on the GABA_A receptor that may provide alternative mechanisms of anticonvulsant activity which might be devoid of tolerance (although this remains to be demonstrated). As the level of understanding of the non-benzodiazepine binding sites, such as the neurosteroid and loreclezole/anaesthetic sites develops [17, 74], so novel approaches to the modulation of the GABA_A receptor will emerge [103] and should encourage further exploration of the pharmacological possibilities inherent in GABA_A subtype-selective modulation.

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Distribution of GABA_A Receptor Subunits in the Human Brain

H.J. Waldvogel, K. Baer and R.L.M. Faull

Abstract GABA_A receptors (GABA_ARs) are pentameric subunit complexes, which surround a central chloride ion channel. GABA_AR subunits are distributed widely throughout the human brain and spinal cord and are activated by the extensive GABAergic inhibitory system that exists throughout the human CNS. These GABA_AR complexes are comprised of combinations of subunits derived from a total of 19 different subunits assembled from a variety of different subunit classes (α_{1-6} , β_{1-3} , γ_{1-3} , δ , ϵ , θ and π). The most widespread subunit in the human brain is the α_1 subunit and this is commonly associated with the β_2 or β_3 subunit and γ_2 subunits. Other α subunits (α_{1-5}) are distributed variably throughout the basal ganglia, hippocampus, brainstem and spinal cord in a complex fashion, and these are associated in varying degrees with β and γ subunits. Less common subunits δ , ϵ , θ and π have a limited distribution, which has not been well documented in human brain. The information described in this chapter is based on published human data using mRNA probes and from immunohistochemical staining using post-mortem human brain sections with antibodies directed against various GABA_A receptor subunits.

1 Introduction

Gamma-aminobutyric acid (GABA) is the most widespread inhibitory neurotransmitter in the central nervous system (CNS). It acts via GABA_A, GABA_B and GABA_C receptors distributed throughout the CNS. This review focuses on

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GABA_A receptor (GABA_AR) subunit localisation in the human brain and cervical spinal cord. GABA_ARs are comprised of pentameric subunit complexes surrounding an inhibitory chloride ion channel. These receptor complexes are comprised of configurations of five subunits assembled from a total of 19 subunits derived from a variety of different subunit classes (α_{1-6} , β_{1-3} , γ_{1-3} , δ , ϵ , θ and π) [1–3]. They are the most widespread inhibitory receptors in the central nervous system and are localised mainly on postsynaptic membranes, but there is also evidence that they occur at presynaptic sites as well as at extrasynaptic sites on dendritic membranes [4]. They are associated with the tubulin-linker protein gephyrin [5] that functions as a postsynaptic organiser molecule for major subtypes of GABA_AR [6], which anchors them into postsynaptic membrane complexes. GABA_ARs facilitate fast-response, inhibitory neurotransmission in the mammalian central nervous system, and the subunit configuration of receptor complexes determines the pharmacological and physiological responses of individual receptors. Extensive research efforts have focussed on the GABA_AR because of its many and varied roles in inhibitory mechanisms in the central nervous system. Dysfunction of GABA_AR responses and mutations in GABA_AR genes leads to various neurological conditions [7], and many major drugs that act primarily via GABA_ARs (e.g. benzodiazepines, barbiturates) are used as sedatives and to treat conditions such as epilepsy, sleep disturbances, anxiety, schizophrenia and others [8]. GABAergic mechanisms are extremely complex and are the subject of numerous anatomical, pharmacological and physiological studies [9–11]. The detailed distribution and the precise localisation of GABA_AR subunits in the brain are therefore critical in determining the composition of individual receptors throughout the brain and to further our understanding of the actions of GABA transmission in the normal functioning brain. It will also aid in understanding the site of action of drugs directed at the various types of GABA_AR complexes. Several excellent detailed *in situ hybridisation* and immunohistochemical studies have been carried out to determine the mRNA localisation and the protein localisation of GABA_AR subunits in the rat and primate brain [12–14]. This has formed the basis for our understanding of the localisation of GABA_ARs subunits in the human brain. Human-specific studies have been carried out principally on post-mortem human tissue [15] to determine the actual localisation of these subunits in the human brain. This chapter focusses on information that has been published in the human brain.

The extensive distribution of GABA_AR throughout the human brain is evidenced by autoradiographic-binding studies using GABA_AR-specific ligands such as the GABA agonist [³H]muscimol, and benzodiazepines such as [³H]flunitrazepam, [³H]RO-151788 [16–19] as well as antibodies to specific subunits and *in situ hybridisation* studies with specific riboprobes to GABA_AR subunits.

2 Subunit Localisation

The following section describes the GABA_A receptor subunit localisation in the various brain regions and is summarised in Table 1.

Table 1 GABA_A receptor subunits in the human brain

Immunoreactivity of GABA _A receptor subunits in the human brain						Other subunits postulated from animal studies [12–14, 42]
	α1	α2	α3	β2/3	γ2	
Cerebral cortex						
Layer 1	+	++++	+++	+++	+++	β1, α4
Layer 2	+++	++++	++++	++++	+++	β1, α4
Layer 3	++++	+++	++	++++	++++	β1, α4
Layer 4	+++	++	++	+++	+++	β1, α4, 5
Layer 5	++	++	+++	+++	+++	β1, α5
Layer 6	++	++	+++	++	++	β1, α4
Basal ganglia						
CN	++	++++	++	++++	+++	α4, δ
GPe	+++	–	++	++	++	α4, δ
GPI	++	–	+	+	+	α4, δ
SNC	+	+	+++	+	++	α4 β1, δ
SNr	++++	++	+++	++++	+++	δ
Hippocampus						
Granular layer	+	++	++	++	+	
Molecular layer	++++	++++	+	+++	+++	α4, 5
hilus	++	+++	++	++	++	
CA1	++++	++	+++	+++	+++	α4, 5
CA2	++	+++	++	+	+	
CA3	+	+++	+	+	+	
S	+++	+++	++	+++	+++	α5, δ
Ph	++++	++++	++	++++	+++	
Midbrain						
SC	+++	+	++	++++	++	
PAG	++++	+	++	+++	++	
R	+++	+	++	+++	++	
SNC	+	+	+++	+	+++	α4, δ
SNr	++++	+	+++	+++	++	β1, δ
Pons and medulla						
Sp5O	+++	+++	++	++++	++	α5, δ
Sp5I	++++	+++	++	+++	++	α5, δ
Sp5C	++++	+++	+++	+++	++	α5, δ
Pr5	++++	++	+++	+++	nd	α5, δ, γ3
Mo5	++	++	+++	+++	nd	α5, δ
Me5	++	++	++	++	+	α5, δ
6	+++	+	++	+++	+++	α5, δ
7	+++	++	++	+++	+++	α5, δ
10	++++	+	++++	++	++	
12	+++	+++	++	+++	++++	α5
SoN	++++	++	+	++	+	
PN	+++	+	+	+++	+++	
RFp	++++	+	+	+++	++	α5, δ, γ3
RFm	++++	++	+	++	+	α5, δ, γ3
LRN	+++	+++	+++	+++	+++	α5, δ, γ3
MVe	+++	++	+	++++	+++	α5, δ, γ3
LVe	++	++	++	++	++	α5, δ, γ3
Ra	+++	+	+++	+++	++	α5, δ, γ3
LC	+	+	++++	+	++++	
IO	+	++++	+++	+++	++	α5, δ, γ3
AON	+	++++	+++	+++	+++	α5, δ, γ3
Cu	++	++	++	+++	++	α5, δ, γ3
LCN	+	++++	+	+	+++	α5, δ, γ3

(continued)

Table 1 (continued)

Immunoreactivity of GABA _A receptor subunits						Other subunits postulated from animal studies [12–14, 42]
	α1	α2	α3	β2/3	γ2	
Gr	++	++	++	+++	+++	
Au	+++	+++	+++	+++	++	
Amb	+++	++	+++	+++	++	
Spinal cord						
lamina I	+	+	+++	+	+	α5
lamina II	++++	++++	+++	++++	++	α5
lamina III	++	++	+++	++	++	α5
lamina IV	+	+	++	+	+	α5
lamina V	+	+	+	+	+	α5
lamina VI	+	+	+	+	+	α5
lamina VII	+++	+++	++	+	++	α5
lamina VIII	+++	++	+	++	++	α5
lamina IX	+++	+++	+++	+++	+++	α5
lamina X	+	+	+	+	+	α5

Abbreviations: ++++ intense immunoreactivity (IR); +++ high IR; ++ moderate IR; + weak IR; – no detectable IR; *nd* not determined; *Au* austral nucleus; *Amb* ambiguous nucleus; *AON* accessory olivary nucleus; *CN* Caudate nucleus; *Cu* cuneate nucleus; *GPe* globus pallidus external segment; *GPI* globus pallidus internal segment *Gr* gracile nucleus; *IO* inferior olivary complex; *LC* locus coeruleus; *LCN* lateral cuneate nucleus; *LRN* lateral reticular nucleus; *LVe* lateral vestibular nucleus; *Me5* Mesencephalic trigeminal nucleus; *Mo5* motor trigeminal nucleus; *PAG* periaqueductal grey; *Ph* Parahippocampal gyrus; *PN* pontine nuclei; *Ra* raphe nuclei; *RFm* reticular formation medulla; *RFp* reticular formation pons; *SC* superior colliculus; *SNc* substantia nigra pars compacta; *SNr* substantia nigra pars reticulata; *SP5I* spinal trigeminal nucleus, interpolar subnucleus; *Sp5O* spinal trigeminal nucleus, oral subnucleus; *Pr5* principal sensory trigeminal nucleus; *R* red nucleus; *S* subiculum; 6 abducens nucleus; 7 facial nucleus; 10 dorsal motor nucleus of the vagus nerve; 12 hypoglossal nucleus

2.1 Cerebral Cortex

Autoradiographic studies have revealed high levels of binding in the human cerebral cortex [19, 20], with the highest levels of binding in the superficial and middle layers of the cortical grey matter. The distribution of GABA_AR subunits in the human cerebral cortex using *in situ hybridisation* studies have established the presence of mRNA for α₁, α₂, α₃, α₄, α₅, β₁, β₂, and γ₂ subunits in the frontal and motor cortical regions [21–24], and α₁, β₂, and γ₂ subunits in the entorhinal cortex [25]. These studies found a variable distribution of the different subunits in different layers of the middle frontal cortex with the highest levels of α₁, β₂, γ₂ in layers III and IV, α₂, β₁ in layers II, III and IV, and α₅ in layers IV, V, and VI. This agrees relatively well with rodent mRNA studies [14] except that α₅ and β₁ levels were lower in the rat. Additional mRNA for subunits α₄, β₃, γ₃ were detected in the rat cortex [14] although these have not yet been probed for in human cortical areas. Immunohistochemical labelling on human neocortex shows that the α₁, β₂, γ₂ subunit levels are highest in layers III and IV, whereas the α₂ subunit is mainly localised to superficial layers and the α₃ subunit is mainly identified in the deeper layers [26]; see Fig. 1c showing high levels of β₂, 3 subunit-immunoreactivity in

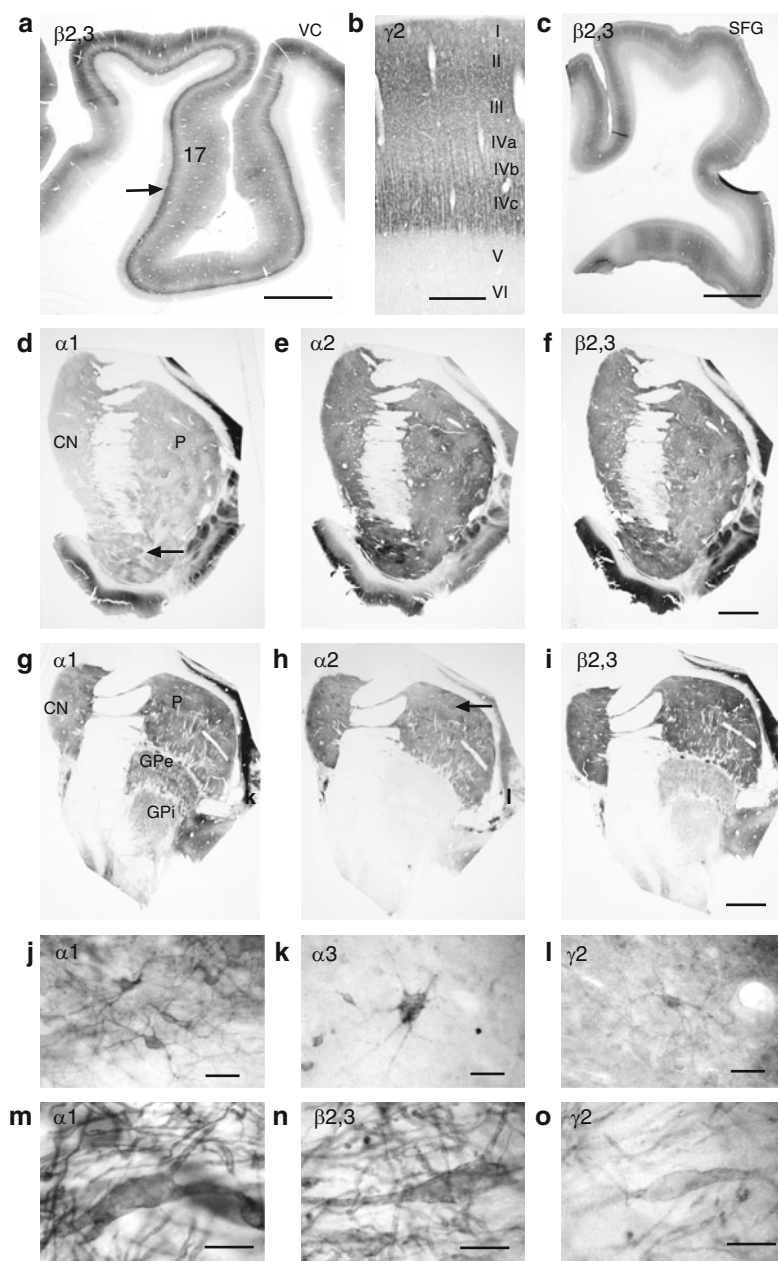


Fig. 1 Representative images of GABA_AR subunits in various regions of the human forebrain. GABA_AR subunit immunoreactivity of (a) $\beta_{2,3}$ subunit labelling of area 17 in the visual cortex with conspicuously staining of a sublaminae of layer IV (arrow), (b) Higher magnification of visual cortex area 17 stained with γ_2 subunit. (c) Superior frontal gyrus (SFG) labelled with $\beta_{2,3}$ subunit which shows high levels of $\beta_{2,3}$ subunits in layers II–III. (d–i) Adjacent sections at the

layers II–III in the superior frontal gyrus. In the visual cortex (area 17) of humans and primates, immunohistochemical studies found that the subunits α_1 , β_2 , γ_2 were the highest in layers II–III, and formed a dense band of labelling in IVA and IVC [27] in the primary visual cortex (Fig. 1a, b for subunits β_2 , γ_2). In rodent studies, these subunits are also the most highly expressed in the equivalent cortical regions [12, 13]. In the frontal and entorhinal cortices, α_2 and α_3 subunits are localised mainly in layers II, III, V, and VI, although differences in intensity of lamina labelling occurs between the two regions [28]; in addition, it is noted that these two subunits are prominent in the initial axon segment of pyramidal neurons. These observations agree with those derived from the rat cortex [29]. The subunits α_4 , α_5 , γ_3 , and δ are also detected in the rodent cortex. The immunohistochemical labelling of α_1 , β_2 , γ_2 subunits were consistently the most common subunits found in all of the different regions of the human cerebral cortex investigated. The receptors are concentrated in dense networks of neuropil label within which pyramidal cells and also interneurons can be distinguished; α_2 subunits were mainly located in the neuropil of each layer. The α_3 subunit mainly outlined pyramidal neurons whose cell bodies were located in layer III and V with their apical dendrites extending to the uppermost layers of the cortex [26].

2.2 Hippocampus

Autoradiographic studies of [^3H] flunitrazepam in the human hippocampus revealed a heterogeneous pattern of binding in the various regions of the human hippocampus with the highest levels in the molecular layer of the dentate gyrus, CA1, CA2, subiculum and entorhinal cortex regions with weaker levels in CA3 and large neurons scattered throughout the hilus [16]. Some of the earliest immunohistochemical studies on GABA_AR subunits in human brain were carried out in the hippocampus. Houser et al. (1988) used antibodies to the α_1 and $\beta_{2,3}$ subunits to detail the labelling of these subunits throughout the hippocampus [30], and these were confirmed in later studies that also included α_2 , α_3 and γ_2 subunits [31, 32]. These studies revealed that the α_1 subunit is the most prominent subunit in the hippocampus but that the $\beta_{2,3}$ and γ_2 subunits produce a similar labelling pattern (Fig. 2l). These subunits were localised most intensely in the molecular layer of the

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Fig. 1 (continued) level of (d–f) human striatum and (g–i) globus pallidus, labelled for subunits α_1 (d, g) α_2 (e, h) and $\beta_{2,3}$ (f, i) subunits. (j) Medium-sized GABA_AR α_1 subunit immunoreactive neurons resembling parvalbumin staining interneurons in the striatum. (k) Large-sized GABA_AR α_3 subunit-ir neuron in the striatum resembling a cholinergic interneuron. (l) GABA_AR γ_2 subunit-ir neuron in the striatum resembling a medium-sized interneuron. (m–o) neurons in the external segment of the globus pallidus labelled with (m) α_1 subunit, (n) $\beta_{2,3}$ subunit and (o) γ_2 subunit. *CN* caudate nucleus; *GPe* external segment of the globus pallidus; *GPI* internal segment of the globus pallidus; *SFG* superior frontal gyrus; *VC* visual cortex. *Scale bars*, a, c, d–f, g–i = 0.5 cm, b = 100 μm , j–l = 20 μm , m–o = 25 μm

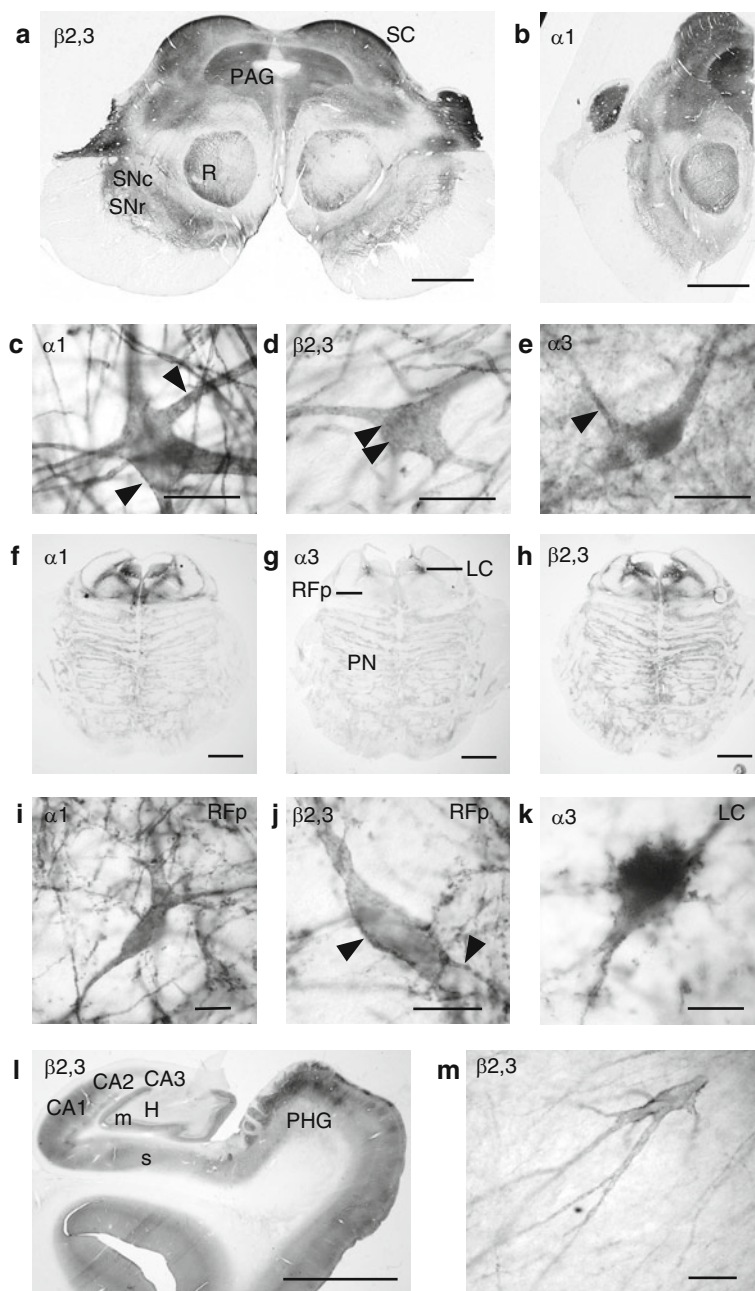


Fig. 2 Representative images of GABA_AR subunits in various regions of the human mid-brain, pons and hippocampus. **(a)** Coronal section of the midbrain at the level of the substantia nigra and red nucleus labelled for $\beta_{2,3}$ subunits. **(b)** Hemisection of the mid-brain at a similar level to **a** labelled for α_1 subunit. **(c)** Neuron and extensive dendritic processes showing surface labelling for

dentate gyrus with lower levels in the hilus and granule cell layer. The highest labelling for these subunits was in the CA1 region with medium labelling in CA2 and virtually no label in the CA3 and hilus. The α_2 subunit was also most abundant in the molecular layer of the dentate gyrus, moderate in the CA1 and relatively high in the CA2, CA3 and the hilus. The α_3 subunit was highly present in the CA1, moderate in CA2 and virtually absent in CA3. At the cellular level, there was a variable staining pattern for the different α subunits. For instance, in CA1, α_1 , α_2 , α_3 , $\beta_{2,3}$ and γ_2 subunits showed a diffuse laminar staining pattern but individual pyramidal cells could be distinguished. In addition, membranes of interneurons were outlined by these subunits. In the CA2, the various α subunits showed different staining patterns at the regional and cellular level; α_1 subunits were detected on both pyramidal cells and interneurons, whereas α_2 subunits showed a diffuse labelling pattern with no clear association with neuronal cell bodies, and α_3 subunit principally outlined the soma and dendrites of pyramidal neurons. In the dentate gyrus, there was a different pattern of staining of the α subunits with low levels of α_1 , high levels of α_2 , with $\beta_{2,3}$ and γ_2 subunits also present. In the hilus, various morphological cell types were observed, and cells immunoreactive for α_1 and α_2 have been described [31], which were identified as mossy cells. In addition, putative interneurons stained for α_1 were localised in the polymorphic layers and sub-granular zone. In the hilus, large multi-polar cells were also observed labelled with $\beta_{2,3}$ subunits (Fig. 2m).

In the dentate granule cell layer, α_1 , α_2 and γ_2 subunits [31] and high levels of $\beta_{2,3}$ subunits [32] were observed in the molecular layer, which contains the dendritic trees of the granule cells. The cell bodies of granule cells were generally weakly labelled for most subunits except for α_3 and $\beta_{2,3}$ subunits. These studies show similarities to the GABA_A α and β subunit distribution in the rat hippocampus with the main differences in the human hippocampus being in the greater diversity and complexity of α_1 subunit positive interneurons in the hilar region and the increased expression of the α_3 subunit in the CA1 and dentate granule cells in the human hippocampus.

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Fig. 2 (continued) α_1 subunit in the SNr. **(d)** A neuron in the SNr labelled for the $\beta_{2,3}$ subunits, which form a lattice-like web of label (*arrowheads*) on the surface membranes. **(e)** High magnification of a pigmented neuron in SNc showing α_3 subunit labelling on surface membranes of the soma and dendrites (*arrowhead*). **(f–h)** Coronal sections through the upper pons at the level of the locus coeruleus labelled for **f**, α_1 subunit, **g** α_3 subunit and **h**, $\beta_{2,3}$ subunits. α_1 subunit immunoreactivity on neurons and dendritic process in the pontine reticular formation. **(j)** high magnification image of a neuron in the pontine reticular formation showing $\beta_{2,3}$ subunit clusters along the surface membranes of the soma and dendrites (*arrowheads*). **(k)** Pigmented neuron in the locus coeruleus outlined by α_3 subunit immunoreactivity. **(l)** Section through the hippocampus labelled with $\beta_{2,3}$ subunit with highest levels of labelling in the molecular layer and CA1 regions. **(m)** Large neuron in the hilar region labelled with $\beta_{2,3}$ subunit outlining the cell body and fine dendritic processes (Fig. 2b from [45]). CN caudate nucleus; CA cornu ammonis; H hilus; LC locus coeruleus; PAG periaqueductal grey; m molecular layer; PHG parahippocampal gyrus; PN pontine nuclei; R red nucleus; RFP pontine reticular formation; SC superior colliculus; s subiculum; SNc Substantia nigra pars compacta; SNr Substantia nigra pars reticulata. Scale bars, **a, b, f–h, l** = 0.5 cm; **c–e, i–k, m** = 20 μ m

2.3 Thalamus

To date, the distribution of GABA_AR subunits of the human thalamus is based mainly on extrapolation of *in situ hybridisation* results of rat, cat and monkey thalamus [14, 33–35]. The most important study in relation to the human thalamus has been the mapping of the mRNA distribution of ten GABA_AR subunits, which was carried out in a series of sections through the monkey thalamus [34]. This has been reproduced in comprehensive reviews of the thalamus [36, 37]. An *in situ hybridisation* study of a smaller number of GABA_AR subunits (α_1 , α_5 , β_2 , γ_2) in the human thalamus found that the distribution in the human was largely similar to that reported in the monkey [36, 38]. All the subunits investigated (α_1 , α_2 , α_3 , α_4 , α_5 , β_1 , β_2 , β_3 , γ_1 , γ_2) were expressed in the monkey thalamus. Their distribution was varied both in their nuclear localisation and their relative density in each nucleus. In general terms, in the monkey, the α_1 , β_2 , γ_2 subunits demonstrated the highest levels throughout the entire thalamus with the other subunits having lesser distribution patterns. The sensory nuclei including the ventral posterior, and the ventral medial and dorsal lateral geniculate body contained the highest levels, especially in the magnocellular layers. The α_4 and α_5 subunits were found in sensory relay nuclei, but not in intralaminar nuclei whereas α_1 , α_2 , α_3 , β_1 , β_3 , γ_1 were relatively high in the intralaminar nuclei. Interestingly, the thalamic reticular nucleus was very low in the expression of most subunits and restricted to mainly α_3 and γ_2 subunits, with a surprising lack of $\beta_{2,3}$ subunits. Immunohistochemical studies carried out in rat [12, 13] generally confirm this overall distribution in the thalamus, but similar immunohistochemical studies are significantly lacking in human thalamus.

2.4 Basal Ganglia

GABA_ARs are localised at high concentrations throughout the basal ganglia [39]. This relates to the fact that the vast majority of intrinsic neurons in the major nuclei and the major projections of the basal ganglia in the basal ganglia circuitry are GABAergic.

2.4.1 Striatum

The majority of neurons in the human striatum are GABAergic projection neurons. In addition, the striatum contains a variety of GABAergic interneurons [40]. Initial autoradiographic studies in human striatum showed high levels of GABA receptor binding [17, 41]. So far, no *in situ hybridisation* studies have been carried out on GABA_AR subunits in the human striatum, although monkey studies show high levels of α_2 , α_3 , α_4 , β_2 , β_3 , δ and moderate γ_2 subunits with low levels of α_1 subunits [42]. Immunohistochemical studies have demonstrated that the subunits α_1 , α_2 , α_3 , $\beta_{2,3}$ and γ_2 are localised in the human striatum [39] and show considerable subunit

heterogeneity in their regional and cellular distribution (see Fig. 1d–f). At the regional level in the human striatum, GABA_AR subunit distribution followed the neurochemical matrix and striosome compartmental organisation defined in previous studies [43, 44]. The striosome compartment contained high levels of the α_2 , α_3 , $\beta_{2,3}$ and γ_2 -subunits while receptors in the matrix compartment contained the α_1 , α_2 , α_3 , $\beta_{2,3}$ and γ_2 -subunits [39]. This was also reflected in the baboon brain where $\beta_{2,3}$ subunits were found in both matrix and striosomes compartments whereas α_1 was only detected in the matrix and generally at a lower density than the other subunits. In the human striatum, six different types of neurons were identified [39]. The major striatal GABAergic projection neurons contained calbindin, and neuropeptides enkephalin and substance P, and they were immunoreactive for subunits α_2 , α_3 , $\beta_{2,3}$, γ_2 . The parvalbumin- and calretinin-positive striatal GABAergic interneurons were associated with the subunits $\alpha_1, \beta_{2,3}, \gamma_2$, (Fig. 1j, l), the large cholinergic interneurons contained high levels of α_3 subunits (Fig. 1k), whereas the NPY/somatostatin interneurons were not labelled for α_1 , α_2 , α_3 , $\beta_{2,3}$ or γ_2 subunits.

2.4.2 Globus Pallidus

The human globus pallidus contains parvalbumin- and calretinin-positive GABAergic projection neurons as well as a sub-population of interneurons. These neurons are associated with subunits α_1 , α_3 , $\beta_{2,3}$, γ_2 [39]. Although no human mRNA studies have been published, these results are consistent with *in situ hybridisation* studies in the monkey globus pallidus [42] showing expression of the ubiquitous α_1 , α_3 , $\beta_{2,3}$, γ_2 with additional subunits also detected including α_2 , α_4 and δ .

Immunohistochemical studies showed high levels of α_1 , α_3 , and moderate levels of $\beta_{2,3}$ and γ_2 subunits with a lack of α_2 subunits in the human globus pallidus. Higher levels of these subunits occurred in the external segment than the internal segment [39] (see Fig. 1g–h for α_1 , α_2 , $\beta_{2,3}$ subunits). The α_1 , $\beta_{2,3}$, γ_2 subunits were distributed along the soma and along the entire length of the dendritic membranes of the large pallidal neurons and interneurons (see Fig. 1m–o for subunits α_1 , $\beta_{2,3}$, γ_2), with the α_3 subunit additionally present in the cytoplasm. GABA_ARs containing the α_1 , α_3 , $\beta_{2,3}$ and γ_2 subunits in their configuration are the dominant receptor type in the globus pallidus. In summary, the subunit composition of GABA_ARs displays considerable regional and cellular variation in the striatum but is more homogeneous in the globus pallidus.

2.4.3 Midbrain

The superior colliculus (SC) was quite heterogeneous in its GABA_AR subunit composition. High levels of α_1 were observed throughout the superior colliculus with more moderate levels of α_2 , α_3 and γ_2 , but high levels of $\beta_{2,3}$ subunit labelling were found in the outer layers of the superior colliculus (Fig. 2a). Similar levels of these antibodies were found in the periaqueductal grey (PAG, Fig. 2a) to that in the

superior colliculus. The red nucleus (R) contained relatively high levels of α_1 , $\beta_{2,3}$, and γ_2 , especially in its periphery. The two regions of the substantia nigra, the GABAergic substantia nigra pars reticulata (SNr) and the dopaminergic substantia nigra pars compacta (SNc), respectively, contained two different receptor combinations. Immunohistochemical studies revealed high levels of α_1 , α_3 , $\beta_{2,3}$, and γ_2 subunits on the large neurons in the SNr [45]. These subunits coated the membranes of the large reticulata neurons, and at high resolution, this coating was observed to have a lattice or web-like structure, which outlined the surface membranes (Fig. 2d). In contrast, the pigmented dopaminergic neurons of the SNc contained mainly α_3 and γ_2 subunits [45, 46]. An additional group of non-dopaminergic neurons labelled for α_1 , α_3 , $\beta_{2,3}$ and γ_2 subunits were also scattered throughout the SNc. Also, a small proportion of the SNc dopaminergic neurons that revealed the SNr-like combination (α_1 , α_3 , $\beta_{2,3}$ and γ_2) were shown to exist [45, 46]. Monkey mRNA studies have detected additional subunit mRNAs in the SNc; for instance, β as well as α_4 and δ subunits, also α_2 and δ subunits mRNAs were detected in the SNr [42].

2.4.4 Brainstem

The brainstem is an extremely complex region comprised of large numbers of motor and sensory nuclei involved in both somatic and autonomic functions of the nervous system. *In situ hybridisation* and immunohistochemical studies have shown a heterogeneous distribution of GABA_AR subunit in the rat brainstem, but the subunit localisation in the human brainstem is far less well characterised.

Our recent immunohistochemical study investigated the distribution of α_1 , α_2 , α_3 , $\beta_{2,3}$, and γ_2 subunits in representative regions of the human pons and medulla oblongata [47]. These studies showed that all of the subunits investigated were heterogeneously distributed throughout the pons and medulla oblongata (Figs. 2f–g, 3a–c; Table 1). In particular, the sensory cranial nerve nuclei (Pr5, SP5O, SP5I and SP5C), the motor cranial nerve nuclei (Mo5, 6, 7, 10, 12), the lateral cuneate nucleus, and solitary nucleus, all showed high levels of α_1 subunit as well as moderate levels of α_3 , $\beta_{2,3}$, and γ_2 subunits with variable levels of α_2 subunit. A similar labelling intensity for these subunits was observed in the reticular formation of the pons and medulla (Figs. 2f–g, 3a–c; Table 1). Exceptions to this general regional pattern of distribution of subunits were the α_2 and α_3 subunits. The α_2 subunit was very high in the inferior olivary complex (Fig. 3b), while the α_3 subunit was very high in the catecholamine neurons of the locus coeruleus (Fig. 2g), the raphe nuclei (Table 1), which was an equivalent pattern to that in the dopaminergic neurons of the SNc (see above, in midbrain). The localisation of the γ_2 subunit was also highly consistent with that of the α_3 subunit in the LC, raphe nuclei and the dopaminergic neurons of the SNc (Table 1). At the cellular level, the various subunits, particularly α_1 , α_2 , α_3 , and $\beta_{2,3}$ subunits, outlined the soma and dendrites of motor neurons in the motor cranial nerve nuclei and ambiguus nucleus, as well as large neurons in the various sub-groups of the pontine and medullary

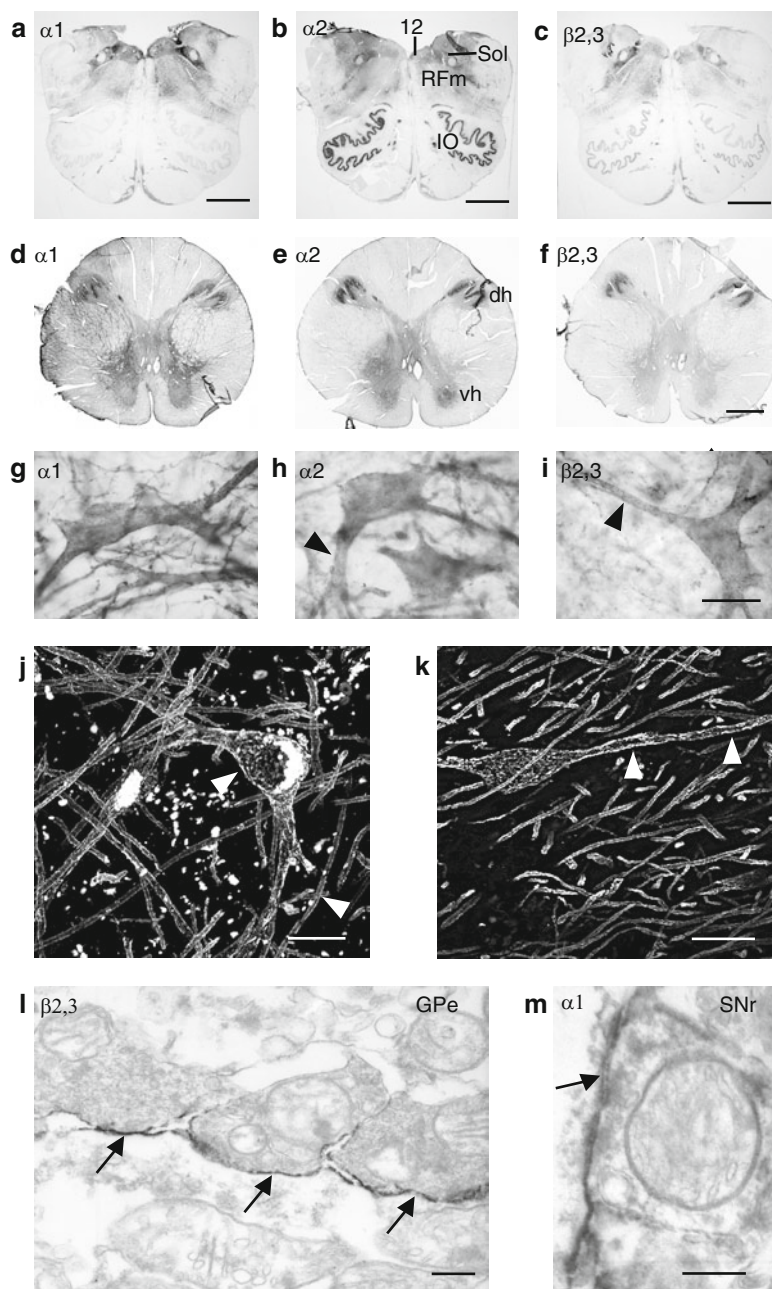


Fig. 3 (a–c) Serial sections of the middle medulla oblongata labelled for, (a) α_1 , (b) α_2 and (c) $\beta_{2,3}$ subunits showing variations in the distribution of these subunits. (d–f) Serial coronal sections of the spinal cord labelled for (d) α_1 , (e) α_2 and (f) $\beta_{2,3}$ subunits. (g–i) Images of motor neurons in the ventral horn of the spinal cord labelled with (g) α_1 , (h) α_2 and (i) $\beta_{2,3}$ subunits. (j) Confocal

reticular formation. Sensory nuclei including the spinal trigeminal nucleus contained high densities of neuropil labelling as well as labelling of smaller neurons.

2.4.5 Spinal Cord

The labelling of subunits in the cervical and lumbar human spinal cord has been carried out with antibodies specific for the α_1 , α_2 , α_3 , $\beta_{2,3}$, and γ_2 subunits [48] (Fig. 3d–f for α_1 , α_2 , $\beta_{2,3}$ subunits). All of these subunits except for GABA_A γ_2 subunit were detected at especially high levels in the “sensory” region of the spinal cord, specifically lamina II, which was evident as an intense band of immunoreactivity in the dorsal horn. GABA_A α_1 and α_2 subunits were also present at moderate levels in the “motor” region of the spinal cord, specifically laminae VIII and IX of the grey matter and especially on neurons in the ventral horn in laminae VIII and IX. GABA_A α_3 and $\beta_{2,3}$ subunits were most prominent in lamina II with high levels in lamina III of the dorsal horn and in lamina IX in the ventral horn (Fig. 3g–i), while γ_2 -IR was the weakest of the GABA_A subunits analysed and showed medium levels of IR in laminae II and IX. The staining patterns for α_1 and $\beta_{2,3}$ were similar in the dorsal horn of the lumbar spinal cord and in the cervical cord [48]. At a cellular level, the GABA_A subunit staining in the dorsal horn formed a dense network of terminals in the neuropil. In the ventral horn, small to medium sized neurons and the majority of large motor neurons were labelled with the α_1 , α_2 , α_3 , $\beta_{2,3}$, and γ_2 subunits.

3 Overall Distribution

Based on the results above, it is evident that GABA_A subunits are heterogeneously distributed throughout the entire human brain and spinal cord in a highly complex fashion. This corresponds with the large numbers of GABAergic projection neurons and interneurons scattered throughout the brain that use GABA as their principal neurotransmitter. Overall, as in rodents, the α_1 and $\beta_{2,3}$ are the most ubiquitously

←

Fig. 3 (continued) laser scanning microscope image of a neuron in the substantia nigra pars reticulata labelled with α_1 subunits. **(k)**, Confocal laser scanning microscope image of a neuron in the globus pallidus labelled with $\beta_{2,3}$ subunits. *Arrowheads* indicate punctate receptor labelling. **(l)**, Ultrastructural localisation of GABA_A $\beta_{2,3}$ subunits (*arrows*) at symmetrical inhibitory synapses in the external segment of the human globus pallidus. **(m)**, Ultrastructural localisation of GABA_A $\beta_{2,3}$ subunits (*arrows*) at a symmetrical inhibitory synapse in the human substantia nigra pars reticulata. (Fig. 3k, courtesy of K Allen, Department of Anatomy with Radiology, University of Auckland) *Dh* dorsal horn; *IO* inferior olivary complex; *Rfm* medullary reticular formation; *GPe* external segment of the globus pallidus *Sol* solitary nucleus, *vh* ventral horn; *12* hypoglossal nucleus. *Scale bars* a–f, 250 μ m, d–h, 25 μ m, l, m = 1 μ m

distributed subunits and are most densely distributed throughout the cerebral cortex, basal ganglia, thalamus, hippocampus, brainstem, and spinal cord. Studies on the distribution of subunits in the human brain are still incomplete and have been mainly restricted to those subunits for which reliable human-specific antibodies and riboprobes are available. A technical challenge is that a large proportion of antibodies and in situ probes that are specific for rodents do not recognise human targets. The distribution of subunits has been intensively studied in animal models with the most complete studies carried out in rodents, and the results of these studies have become the foundation for our knowledge of the subunit distribution in the human brain. From a comparative study of the distribution of GABA_AR subunits outlined above, it is apparent that the distribution pattern in the human brain and spinal cord apparently follows an overall pattern that is very similar to what is seen in rodent and sub-human primate studies.

3.1 Cellular Distribution of GABA_A Receptor Subunits

At the cellular level in the human brain and spinal cord, certain subunits are associated with particular sub-types of neurons that are defined by specific cell markers and neurotransmitters. In particular, the α_1 subunit is highly expressed on the membranes of interneurons throughout the brain, which use GABA as their transmitter and often contain calcium-binding proteins such as parvalbumin, calretinin and calbindin [39]. These interneurons are distributed mainly throughout the cerebral cortex, basal ganglia and hippocampus. Neurons identified as GABAergic projection neurons such as the output neurons of the basal ganglia located in the globus pallidus and substantia nigra pars reticulata have high levels of the α_1 subunit. The receptors form “hotspots” of label along dendritic membranes when viewed with high resolution confocal laser microscopy (Fig. 3j, k) [45].

The α_2 subunit is very highly expressed throughout the cerebral cortex, hippocampus, and striatum and in the inferior olivary complex. Throughout the cerebral cortex, the α_2 subunit is distributed presumably on pyramidal neurons: in the hippocampus, it is mainly on CA1–CA3 pyramidal neurons as well as the molecular layer and in the hilus; in the striatum, it is mainly located on medium spiny projection neurons, and in the inferior olivary complex, it is high on olivary neurons, which project to the cerebellum. In the spinal cord, it is especially prominent in the sensory region of the dorsal horn, i.e., lamina II.

The α_3 subunit is restricted mainly to pyramidal neurons of the cerebral cortex, cholinergic interneurons in the striatum, and dopaminergic neurons of the substantia nigra pars compacta and nor-adrenergic neurons of the locus coeruleus. The α_3 subunit is commonly associated with monoaminergic and cholinergic neurons [12, 49, 50] and has been implicated in muscle relaxant activity and thalamic oscillations via the reticular nucleus [51].

The $\beta_{2,3}$ subunits are highly distributed throughout all the regions associated with α_1 , α_2 and α_3 subunits with the exception of α_3 subunits localised in the

mono-aminergic neurons of the SNc and locus coeruleus where the $\beta_{2,3}$ subunits were not detected. The γ_2 subunit distribution is relatively highly correlated with the pattern of all of the above subunits although the expression level is often relatively low. The cellular localisation of α_4 , α_5 and δ subunits have so far not been resolved to specific cell types.

3.2 Ultrastructure

The α_1 and $\beta_{2,3}$ subunits have been examined ultrastructurally in the human spinal cord, globus pallidus (Fig. 3l) [15, 48] and in the human substantia nigra (see Fig. 3m) in human tissue as well as in non-human primate [52, 53]. In these studies, α_1 and $\beta_{2,3}$ subunits were localised at symmetrical synapses on large and small dendrites in the globus pallidus and in the dorsal horn, as well as at axoaxonic synapses in the dorsal horn. In the globus pallidus and substantia nigra pars reticulata, these synapses were on dendrites and soma of large neurons. The receptor subunit labelling was localised at synapses adjacent to presumed striato-pallidal and striatonigral terminals as well as at larger extra-striatal terminals. In addition, receptors were labelled on the surface membranes at extra-synaptic sites (Fig. 3l) and on both pre and postsynaptic membranes. This organisation was also confirmed in the baboon and other animal studies using immunogold labelling [52–54]. In addition, recent studies in animals have provided evidence of pre-synaptic and extra-synaptic GABA_ARs in rodent CNS [55, 56].

3.3 Subunit Configurations

The most common GABA_AR subunit in the mammalian brain is the α_1 subunit [12, 57]. It is commonly found in the configuration of α_1 , $\beta_{2,3}$, γ_2 [58, 59], which forms the benzodiazepine sensitive type I receptor [60] also classified as the A1a GABA_AR sub-type [1]. Based on our results and those of animal studies [61], it is therefore a highly likely configuration for GABA_AR in the human brain. This receptor type is also rendered insensitive to diazepam with point mutations in the α_1 subunit gene [62]. The presence of an α_3 subunit within this configuration is also possible based on observations that the α_3 , β_2 γ_2 combination is recognised as the A3a sub-type of GABA_AR [1]. Additionally, the α_1 and α_3 subunits can be found together as $\alpha_1\alpha_1$, $\alpha_3\alpha_3$, $\alpha_1\alpha_3$ combinations in the rodent brain [63]. Extrapolation of the results of studies on the distribution of subunits in the human brain described so far with similar more extensive studies in other species would indicate that the major receptor types postulated to exist in the human brain are $\alpha_{(x)}\beta_{(x)}\gamma_{(x)}$ comprising the combinations $\alpha_1\beta\gamma_2$, $\alpha_2\beta\gamma_2$, $\alpha_3\beta\gamma_2$, $\alpha_4\beta\gamma_2$, $\alpha_5\beta\gamma_2$, where β -subunits are most likely to be β_2 and β_3 with β_1 the least common subunit [64]. Another common combination includes $\alpha_3\gamma_2$ lacking β_2 or β_3 , which is localised to dopaminergic or

adrenergic cell types. Evidence also exists that the combination $\alpha_4\beta_{(x)}\delta$ is a highly likely configuration throughout many regions of the brain especially including the thalamus (see Table 1). Other combinations of subunits are also highly possible, but until more definitive evidence exists in human brain, these will not be discussed further here; several extensive reviews have dealt with these possibilities in animal studies in detail [65].

One significant feature of GABA_AR subunits in human brain has been their high degree of plasticity in neurodegenerative diseases. In particular, the subunits α_1 , $\beta_{2,3}$, γ_2 all up-regulate to a high degree in the globus pallidus of Huntington's disease presumably as a result of loss of GABAergic striatal projection neurons [66]. In temporal lobe epilepsy, there is selective reduction of α_3 subunits in temporal cortex and up-regulation of α_2 subunits in the hippocampus [26, 31]. In schizophrenia, the expression of α_1 subunit mRNA is increased markedly in the prefrontal cortex [24].

3.4 GABA_A Receptors and Sleep

With regard to the association of GABA_ARs and the features of sleep, there is a large body of evidence linking GABA and GABA_ARs in many aspects of sleep. Sleep is organised into various stages and can be divided into REM and non-REM sleep in a very complex fashion combining a range of anatomical regions and pathways with a variety of physiological and pharmacological mechanisms [67, 68]. Non-REM sleep is interrupted by periods of REM sleep sometimes called paradoxical sleep, which is characterised by mixed frequency bursts of activity and dreaming, and also complete inhibition of skeletal muscle tone [69]. Several brain regions are involved in the regulation of sleep including nuclei in the hypothalamus, brainstem regions such as the pontine and medullary reticular formation, raphe nuclei, locus coeruleus and adjacent regions, the thalamic reticular nucleus and regions of the cerebral cortex. Different parts of the sleep cycle are controlled by different brain areas and pathways. A complex interaction of noradrenergic, adrenergic, serotonergic, cholinergic, glutamatergic and GABAergic neurons in these regions are involved in the control of sleep [67, 68]. GABA and GABA_ARs are also known to be intimately involved in the processes of sleep in various regions of the brainstem. REM sleep is regulated primarily by nuclei located at the junction of the mid brain and pons, particularly the nucleus reticularis pontis oralis (PnO) located in the pontine reticular formation (RFp, Fig. 2f) [67]. The PnO is critical for induction of REM sleep and GABAergic inhibition of cholinergic neurons via GABA_ARs is one of the principal mechanisms operating in the PnO [70, 71].

Several other brainstem nuclei that are associated with the control of various parts of the sleep cycle are controlled by the release of GABA; for instance, noradrenergic neurons in the locus coeruleus are tonically inhibited by GABA during slow wave sleep and REM sleep [72, 73]. In addition, GABAergic inhibition of serotonergic neurons in the dorsal raphe nucleus is key in controlling REM sleep [73]. One of these GABAergic sources may be the inhibitory neurons in the VLPO

of the hypothalamus, which project to various regions of the arousal system including tuberomammillary nucleus (TMN), locus coeruleus and dorsal raphe nucleus [74]. In addition, thalamocortical oscillations are involved in non-REM sleep induction. These are controlled through GABAergic innervation of thalamic relay neurons by the thalamic reticular nucleus, which are in turn innervated by various brainstem projections [75]. The GABAergic control of sleep pathways in the sleep related nuclei described above are thought to operate through GABA_ARs as their effects can be blocked by GABA_AR antagonists, for instance, in the locus coeruleus [72]. Sedatives and hypnotics are known to act through GABA_ARs containing the α_1 subunit. In addition, sleep action is also influenced by α_2 , α_3 and α_5 subunits [76]. Anaesthetics are also known to act on receptors containing α_2 , α_3 and β_3 subunits [77, 65]. In the human brain, GABA_AR α_1 subunits were found highly localised in most of the sleep-related areas of the brain including the reticular formation of the pons, which contains the PnO, and throughout the thalamus and cortex. Interestingly in the raphe nucleus, locus coeruleus and thalamic reticular nucleus, it is the $\alpha_3\gamma_2$ combination that is most prevalent. The combination $\alpha_4\beta_3\delta$ is also considered a receptor configuration associated with sleep and is a likely combination in several brain regions such as thalamus, cortex and basal ganglia [65]. The complex relationships of the sleep-related nuclei and their relationship with GABA and GABA_AR subunits still remain to be clearly elucidated. It will be critical to determine the subunit composition of individual neurons in the reticular formation of humans that are equivalent to the neurons which have been identified as being critical with sleep functions in rodent studies.

The regional and cellular distribution of GABA_AR subunits in the human brain is still incomplete. For instance, detailed localisation of subunits at the protein level has not yet been published for the cerebellar cortex or the thalamus in human brain.

It is clear that GABA_A receptors play a critical role in sleep control mechanisms, and further work is needed to contribute to our understanding of the complexity of GABA and GABA_A receptor subunits in sleep and waking mechanisms in human brain function.

Acknowledgements This work was supported by grants from the Neurological Foundation of New Zealand and the Health Research Council of New Zealand. We thank the Neurological Foundation of New Zealand Human Brain Bank for providing the human brain tissue used in these studies. We also thank the Biomedical Imaging Research Unit (BIRU) in the Department of Anatomy with Radiology, University of Auckland for their expert assistance and allowing the use of their facilities. KB is grateful for support from the British Royal Society.

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Pharmacokinetic Determinants of the Clinical Effects of Benzodiazepine Agonist Hypnotics

David J. Greenblatt

Abstract Beginning with nitrazepam in the early 1960s, the class of drugs acting as benzodiazepine (BZ) receptor agonists has become the principal therapeutic option for the pharmacologic treatment of insomnia. With the development of analytic techniques to measure plasma drug concentrations, along with the more general application of pharmacokinetic methodologies, understanding of the pharmacokinetic determinants of hypnotic drug action has progressed along with the development and clinic use of a series of BZ agonist hypnotics. Absorption rate is a key determinant of the onset of drug action and the effectiveness of a hypnotic in shortening sleep latency. Rapid absorption implies high maximum plasma concentrations and short times to reach the maximum, thereby increasing the probability of rapid-onset effects. Elimination half-life is a determinant of the duration of action and a hypnotic drug's efficacy in prolonging sleep duration and reducing early morning awakening. However, half-life may not directly predict duration of action, since some rapidly absorbed drugs may have action terminated by distribution rather than elimination. In any case, attaining a duration of action that is long enough to sustain sleep but not so long as to produce residual daytime sedation is a difficult objective. Dosage is a major determinant of pharmacodynamics that is often overlooked. Higher doses produce effects of more rapid onset, greater intensity, and longer duration. During multiple dosage with hypnotics, drug accumulation will occur if the drug's elimination half-life exceeds the dosage interval (24 h). This may lead to cumulative daytime sedative effects. In contemporary practice, short half-life BZ agonist hypnotics are most commonly prescribed. These have a low risk of daytime sedation and accumulation, but they must be tapered at the end of treatment to avoid rebound and other discontinuation effects. Ongoing pharmaceutical

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research has the objective of developing drug delivery innovations that can optimize hypnotic drug action by regulation of release and systemic exposure to short half-life BZ agonists.

1 Introduction

Patients with subjectively defined insomnia report one or more specific problems such as taking too long to fall asleep, waking up repeatedly at night, early morning awakening, inadequate total sleep time, and feeling “not rested” when they wake up [1–5]. The principal consequence is daytime sleepiness, leading to mood disorders, poor work performance, and generally reduced quality of life. A consequence of public health concern is impaired driving performance and falling asleep while driving, leading to automobile accidents [6–12].

The pharmacologic management of insomnia has always presented a dilemma in clinical therapeutics. In many patients who seek medical treatment for insomnia, sleep difficulties as such accompany other primary medical or psychiatric disorders. Examples include congestive heart failure, chronic pain, depression, use of alcohol or other substances, and excessive caffeine use. Insomnia may also result from environmental or situational factors, such as shift work, travel across time zones, personal stress or crises, and noise or other disruption. Insomnia due to medical/psychiatric disorders or environmental/situational factors may be improved or even eliminated when the underlying problem is addressed. Still, many cases of insomnia seen by treating physicians do not have an evident underlying cause, and the sleep disorder exists as a primary or autonomous entity.

Balancing the benefits and drawbacks of pharmacologic management of insomnia is not a straightforward task for physicians and patients. Undertreatment or nontreatment of insomnia implies that the sleep disorder will continue, along with the sequelae of daytime sleepiness and nonoptimal quality of life. Appropriate pharmacologic management has the potential to improve sleep, reduce daytime sleepiness, improve performance, and enhance quality of life. Yet, an important and well-recognized drawback of hypnotic drug treatment is the potential for residual daytime effects – often termed “hangover” – thereby counteracting the benefits of improved sleep at night [13]. This is a subtle balance, and it may be tempting to overlook the “benefit” end and focus only on the “risk.” For example, some (but not all) epidemiologic studies report an increased hazard of automobile accidents among patients taking benzodiazepine hypnotics and anxiolytics [14–22], inviting the conclusion that hypnotic drugs should not be used by patients planning to operate an automobile [23, 24]. What the studies unfortunately do not do is evaluate the risk of hypnotic drug use *among patients with insomnia* in relation to the risk of no treatment *among comparable patients with insomnia* – a group known to have a risk of falling asleep at the wheel due to their

sleep deprivation. A corollary area of limitation in the medical literature includes the controlled studies of residual hypnotic drug effects in healthy volunteers without insomnia, who by definition do not see themselves as having daytime drowsiness or impaired performance due to (nonvoluntary) sleep deprivation. Drug–placebo differences in hangover effects found in noninsomniac volunteer studies cannot be assumed to extend to patients with insomnia and daytime sleepiness. Finally, a number of studies evaluate residual effects of hypnotics using driving simulators, or a model of “actual” driving performance [13–15]. These study paradigms have the limitation of lacking the potentially lethal consequences of error. Even the actual automobile operation models include protections against excessive risk that any clinical research protocol must incorporate. This is unlike real-life “unprotected” driving, in which slow reactions, inattention, or a performance mishap could be fatal. The “vigilance-enhancing” effects of real driving, which may counteract the effects of sleep deprivation or drug hangover [25], raise questions about the extent to which even the most sophisticated and realistic laboratory testing protocols can be extrapolated to actual life tasks in patients with insomnia.

The above notwithstanding, there are clinically important differences among available benzodiazepine (BZ) agonist hypnotic agents in the onset, intensity, and duration of pharmacodynamic effects. These differences are *not* likely to be attributable to variations among the available drugs in the qualitative character of binding to the gamma-aminobutyric acid benzodiazepine (GABA-BZ) receptor complex. The various drugs (and their metabolites) may differ in the quantitative affinity of binding, but the nature of the drug–receptor interaction is similar or identical. Clinical differences are due to pharmacokinetic properties, and to the effects of dosage [26–34]. This chapter reviews the pharmacokinetic determinants of hypnotic drug action, along with a discussion of pharmacokinetic properties of individual drugs.

2 Historic Perspective

The benzodiazepine era officially began in 1960 with the introduction of chlordiazepoxide (Librium) in the US [35]. Diazepam (Valium) appeared a year later, in 1961. At that time, a number of older nonbenzodiazepine hypnotics were available for the treatment of insomnia, and many of these were widely prescribed (Table 1) [36, 37]. However, these older sleeping medications had important drawbacks, including: (1) A relatively narrow therapeutic index, such that intentional overdosage posed a serious hazard of life-threatening central nervous system (CNS) and respiratory depression; (2) A risk of additive CNS depression when combined with alcohol; (3) A hazard of abuse and dependence; (4) A risk of causing drug interactions through microsomal enzyme induction (by barbiturates and glutethimine) or displacement of protein-bound acidic drugs from plasma albumin (by trichloroacetic acid, the metabolite of chloral hydrate and trichloroethyl phosphate).

Table 1 Representative hypnotic drugs used in the United States in the prebenzodiazepine era

Chloral hydrate
Ethchlorvynol
Glutethimide
Methaqualone
Methyprylon
Pentobarbital
Secobarbital
Trichloroethyl phosphate

Nitrazepam (Mogadon) was the first BZ derivative specifically indicated for the treatment of insomnia, and was introduced in the United Kingdom in the 1960s [35, 38]. The most striking consequence was a major reduction in the incidence of life-threatening or fatal intoxication following overdosage with nitrazepam compared to barbiturates [39–41]. The neurochemical mechanisms of the enhanced safety of nitrazepam and other BZ derivatives was later attributed to the differing effect of BZs and barbiturates on the GABA-BZ receptor-linked chloride channel [42]. Nitrazepam was also considered to be less abusable than the older hypnotics, and also to have a much lower or negligible propensity to participate in pharmacokinetic drug interactions.

Flurazepam (Dalmane) was the first BZ hypnotic to be introduced in the United States in 1970 [43, 44]. Like nitrazepam in the UK, flurazepam became widely prescribed in the US, and appeared to follow the general profile of nitrazepam and other BZs in terms of relative safety with overdosage, low abuse liability, and noninvolvement in drug interactions. By the mid1970s, BZs, including flurazepam, were at or near the top of the most commonly prescribed drugs in the United States [45–47]. Diazepam (Valium) earned the colloquial name “Vitamin V.” At about the same time, a “backlash” against BZs also began, first in the UK, then in the US [48–50]. The targets of allegations included: the pharmaceutical manufacturers of BZs, accused of seeking excessive profit by overpromoting products that were not really medically needed; practicing physicians, encouraged by drug advertising, who overprescribed BZ derivatives; patients, who too readily asked for chemical solutions to stresses and problems of everyday life; and regulatory agencies, who failed to heed warning signs about the hazards of BZ abuse and dependence. By 1980, BZ hypnotics and anxiolytics continued to be widely prescribed, but usage prevalence was dampened by controversy in the medical literature regarding the possibility of benzodiazepine tolerance and dependence. It is ironic that most of the “anti-BZ” rhetoric was based on anecdotal observations and uncontrolled reports, whereas well-controlled clinical and epidemiologic studies did not verify a pattern of extensive BZ misuse or dependence [51–55].

The triazolobenzodiazepines – triazolam and alprazolam – became prominent in clinical therapeutics during the decade of the 1980s, and marked a shift in usage patterns away from long half-life BZs (such as diazepam and flurazepam) in favor of the shorter half-life derivatives including the triazolo compounds and lorazepam. Triazolam itself came under attack from opponents in the scientific community and in the lay press, with allegations that this drug had a unique profile of

risks and adverse effects not shared by other BZs [56]. Again, these allegations were based on anecdotal and uncontrolled reports, encouraged and sensationalized by the media [56, 57]. Controlled studies and scientifically valid epidemiologic work failed to validate any unique properties or hazards of triazolam (Halcion) [57]. An extensive review by the Institute of Medicine in 1997 [58] concluded:

The data from premarketing clinical trials, postmarketing studies, and the published literature do not support clearly the existence of a unique profile or syndrome of adverse events associated with Halcion relative to those associated with other drugs of its type. Furthermore, reanalysis of 25 parallel-group, placebo-controlled studies and a review of the published literature did not provide clear evidence of a greater risk of adverse events associated with Halcion relative to the risk of adverse events associated with comparator drugs of its class.

Despite the scientific exoneration, the clinical use of triazolam declined substantially. Zolpidem, having an imidazopyridine structure, emerged in the 1990s as a short half-life hypnotic [59–65], and is now the most widely prescribed hypnotic drug in the world. In neurochemical models, zolpidem appears to have relative selectivity for specific BZ receptor subtypes, as opposed to the nonselectivity of the older BZ agonist hypnotics [66–76]. This distinction is emphasized in pharmaceutical materials promoting zolpidem, but it has been difficult or impossible to demonstrate that BZ receptor subtype selectivity confers any identifiable clinical benefit. Zopiclone – racemic zopiclone in and eszopiclone in the US – is a cyclopyrrolone derivative also identified as a “non-BZ” hypnotic, yet it acts at the same GABA-BZ receptor [77–79]. The half-life of zopiclone and eszopiclone falls in the range of 4–8 h. Zaleplon, a pyrazolopyrimidine derivative, has a half-life of approximately 1 h, and is the third of the “Z hypnotics” [59, 80–83].

Table 2 shows selected prescription hypnotic drugs used in the United States, categorized according to generic or brand status, and average cost per prescription. A number of drugs (triazolam, flurazepam, zaleplon, ramelteon) were not prescribed sufficiently frequently to make the list. Net expenditures for these hypnotics in 2008 totaled nearly \$2.5 billion, and the number of prescriptions was nearly 50 million. There is a striking difference between generic and brand-name drugs in extent of use and cost. Generic drugs (immediate-release zolpidem, temazepam)

Table 2 Prescriptions and expenditures for selected hypnotic drugs in the United States, 2008

Drug	Rx number (thousands)	\$Cost (thousands)	\$Per Rx
Zolpidem-CR (Ambien-CR)	7,214	865,719	120.01
Eszopiclone (Lunesta)	5,622	771,019	137.14
Zolpidem (generic)	28,324	742,325	26.21
Temazepam (generic)	7,911	84,727	10.71
Total	49,071	2,463,790	

Source: Verispan VONA

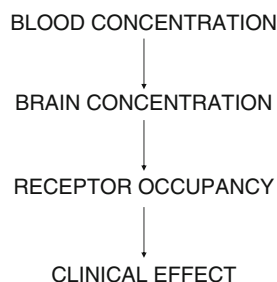
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are most commonly prescribed and are far less expensive. The brand-name drugs (controlled-release zolpidem, eszopiclone) are less prescribed and much more expensive. Pharmaceutical promotion focuses exclusively on the more expensive brand-name drugs. A critical and unanswered question faced by practicing physicians, patients, and insurance plans is whether the expensive brand-name hypnotic agents provide a substantive benefit in terms of efficacy or safety compared to the inexpensive generics to warrant the higher dollar cost.

3 Pharmacokinetic Determinants of Hypnotic Drug Action

Linking the pharmacokinetics of hypnotics to the time-course of their clinical action assumes a connection between plasma concentration, CNS concentration, GABA-BZ receptor occupancy, and pharmacodynamic effect (Fig. 1). This cascade has been documented in a number of experimental animal studies [84–91]. The linkage of drug concentration to pharmacodynamic action has also been well-described in controlled clinical studies using effect measures such as subjective or objective sedation, psychomotor performance proficiency, reaction time, and electroencephalographic (EEG) activity in the “beta” frequency range [89–91]. The outcome of these studies may encourage the conclusion that there is a direct and predictable connection between plasma drug concentration and the action of the drug in terms of inducing and maintaining sleep, as well as the risk of sedative effects extending into the waking hours the next day. These relationships are consistent with indirect evidence, but there is no direct evidence for any hypnotic drug indicating a specific plasma level threshold or range needed to induce sleep, or a level/range below which sleep is no longer sustained. It also cannot be assumed that the plasma concentration associated with the induction of sleep shortly after drug dosage is equal to the plasma level below which awakening occurs at some later time in the morning. Nonetheless, some general conclusions can be drawn regarding probabilities of association: (1) Rapid drug absorption is likely to have a favorable effect on sleep latency; (2) Sustained plasma levels at the other end of the dose interval are likely to prolong sleep duration; (3) Plasma levels persisting into

Fig. 1 Sequence of events linking systemic blood concentration of benzodiazepine agonists to eventual pharmacodynamic effect



the morning hours are likely to produce hangover; (4) All drug effects are enhanced and/or prolonged at higher doses.

3.1 Absorption Rate and Onset of Action

The assumed linkage of hypnotic drug absorption rate, the onset of sedative activity, and the probability of shortening sleep latency is reasonable and well justified based on available clinical and scientific data. Rapid absorption implies a relatively high maximum plasma concentration (C_{\max}) and a short interval between dosage and the time of maximum concentration (T_{\max}). Conversely, slow absorption yields a lower C_{\max} and a longer T_{\max} . In many cases, it is possible to determine a first-order absorption rate constant (K_A), having units of 1/time. This can be used to derive an absorption half-life, calculated as $(\ln 2)/K_A$, representing the time necessary for absorption to be 50% complete. A large value of K_A means a short absorption half-life, consistent with rapid absorption.

The most straightforward approach to understanding plasma concentrations of hypnotic drugs applies the one-compartment pharmacokinetic model (Fig. 2) [92]. The drug is assumed to pass from the gastrointestinal tract to the systemic circulation, via a first-order process characterized by K_A . First-order drug elimination proceeds according to an elimination rate constant (K_E), which can be used to calculate the elimination half-life $[(\ln 2)/K_E]$. The hypnotic drug is assumed to equilibrate rapidly between plasma and the GABA-BZ receptor site in brain.

For a hypothetical drug having an elimination half-life of 3 h ($K_E = 0.231/\text{h}$), the shape of the plasma concentration curve will depend on the rate of absorption. The larger the value of K_A , the more rapid the absorption, the higher the value of C_{\max} , and the shorter the value of T_{\max} (Fig. 3). C_{\max} and T_{\max} each are predictably

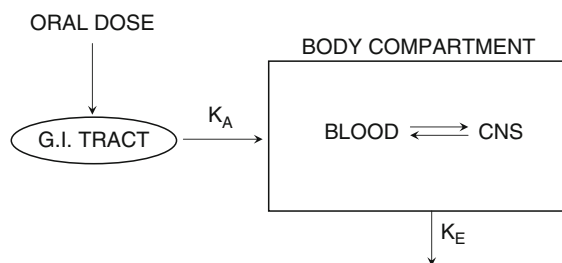


Fig. 2 Schematic representation of the one-compartment pharmacokinetic model. Orally administered hypnotics are absorbed from the gastrointestinal into the “body compartment” as a first-order process having a rate constant K_A . There is rapid equilibration among all tissues in the body compartment. This includes the circulating blood, which itself rapidly equilibrates with the central nervous system (CNS). Drug elimination or clearance occurs from the body compartment as a first-order process with rate constant K_E

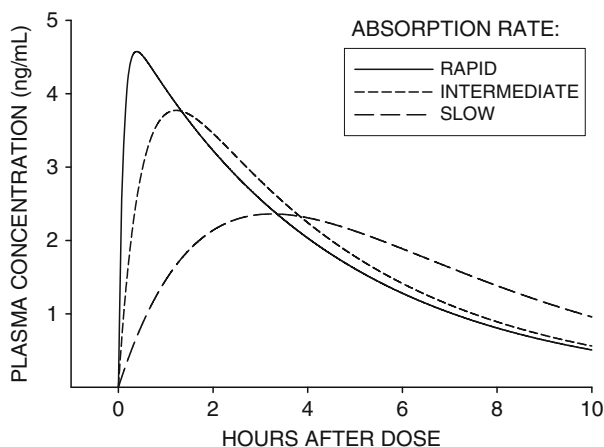


Fig. 3 Effect of absorption rate on the shape of the plasma concentration curve after oral administration of a drug. It is assumed that the drug's behavior is consistent with the scheme shown in Fig. 2, and that the drug has an elimination half-life of 3 h ($K_E = 0.231/\text{h}$). The three different rates of absorption shown are: rapid ($K_A = 10/\text{h}$); intermediate ($K_A = 2/\text{h}$); and slow ($K_A = 0.4/\text{h}$). The three curves are constructed such that the only difference among them is the absorption rate. In all three cases, the net amount of drug absorbed into the circulation is the same (based on the total area under the plasma concentration curve from time zero to infinity)

related to K_A in this way (Fig. 4), and there is an inverse relationship between C_{\max} and T_{\max} (Fig. 5). In the (hypothetical) event that a specific concentration threshold needed to be crossed for a hypnotic drug to effectively reduce sleep latency, rapid absorption increases the likelihood that this threshold is crossed, and crossed rapidly.

In general, hypnotic drugs used in clinical practice are absorbed rapidly enough to have efficacy in shortening sleep latency.

3.2 Elimination, Distribution, and Duration of Action

It makes intuitive sense to associate duration of drug action with elimination half-life. The longer the elimination half-life, the more likely it is that “sedating” drug concentrations in plasma will be sustained until the intended completion of the sleep cycle – for example, 7–8 h after dosage (Fig. 6). On the positive side, this could mean efficacy in prolonging sleep duration and preventing early morning awakening. On the negative side, levels sustained into the daytime hours when alertness and wakefulness are intended could imply unwanted hangover.

However, this relationship may be complicated by drug distribution. Rapidly absorbed drugs that are highly lipophilic may have a phase of declining plasma concentrations immediately following the absorptive peak, attributable to tissue

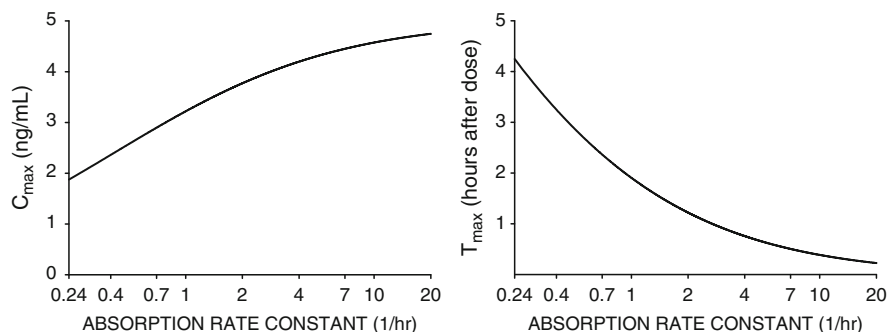
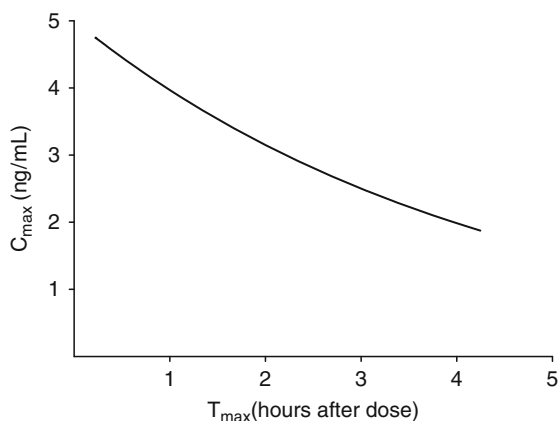


Fig. 4 For the same drug discussed in Fig. 3, the relation of absorption rate constant (x-axis, logarithmic scale) to the maximum plasma concentration (C_{\max} , left graph) and time of maximum concentration (T_{\max} , right graph). As the absorption rate constant becomes larger (indicating more rapid absorption), C_{\max} becomes higher and T_{\max} becomes shorter

Fig. 5 For the same drug discussed in Figs. 3 and 4, the relation of C_{\max} to T_{\max} . As T_{\max} becomes longer (indicating slower absorption), C_{\max} becomes smaller



distribution rather than clearance (Fig. 7). This may cause the drug to have a shorter duration of action than would be predicted based on half-life alone. The extent to which distribution contributes to terminating drug action is not easily predictable, but in any case is a factor in disrupting a straightforward relationship between hypnotic drug half-life and duration of action.

3.3 The Effect of Dose

Dosage influences all phases of a drug's plasma concentration profile. Higher dosage implies higher concentrations at all points in time after drug administration, and is likely to shorten the time to onset of action, produce higher maximum effects,

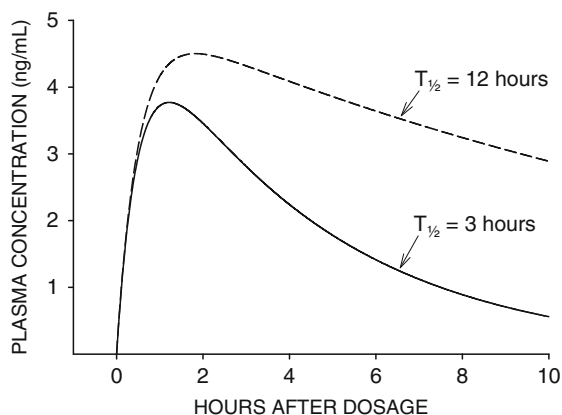


Fig. 6 The effect of elimination half-life ($T_{1/2}$) on the shape of the plasma concentration curve. Two different drugs are shown, having $T_{1/2}$ values of 3 h (solid line) or 12 h (dashed line). The rate of absorption is the same in both cases ($K_A = 2/h$). Note that for the drug with $T_{1/2}$ of 12 h, plasma concentrations are substantially higher at the end of the sleep cycle (8–10 h after dosage). This drug is more likely to sustain sleep throughout the intended sleep cycle, but also more likely to produce daytime residual effects (hangover)

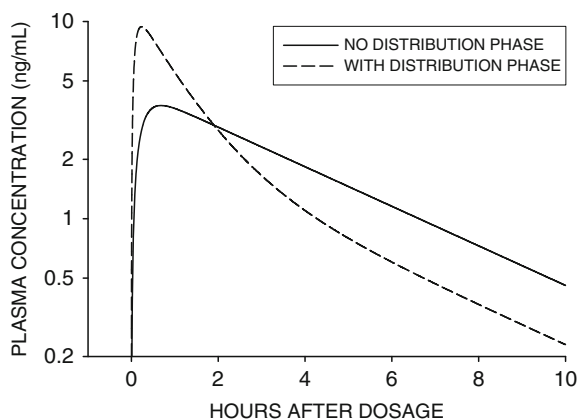
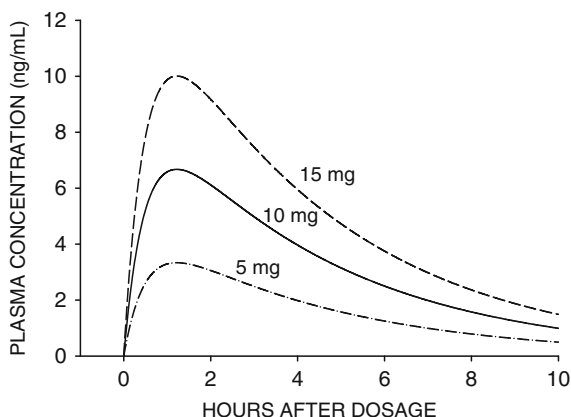


Fig. 7 Plasma concentration curves for two drugs having the same elimination half-life ($T_{1/2} = 3$ h). Note that the plasma concentration scale (y-axis) is logarithmic. The drug shown as having no distribution phase has behavior consistent with the model in Fig. 2. The other drug (dashed line) is very rapidly absorbed, and its behavior is no longer consistent with the one-compartment model. In the period after the absorption peak, there is a phase of drug distribution lasting up to about 4 h after dosage. This distribution phase may actually contribute to terminating drug action, and the elimination half-life may not accurately reflect the duration of clinical activity. Note that plasma levels at 8–10 h after dosage for the drug undergoing distribution (dashed line) are lower than for the drug without a distribution phase (solid line), even though the two drugs have the same elimination half-life. The curves have been drawn such that the net amount of drug reaching the circulation (based on the total area under the plasma concentration curve) is identical for the two drugs

Fig. 8 Plasma concentration curves for the same hypothetical drug given at three different dosage levels. The drug is assumed to be consistent with the model in Fig. 2. The elimination half-life is 3 h



and prolong the duration of effect (Fig. 8). Given in a high enough dose, even a short half-life hypnotic may have an extended duration of action. It is important to note that the relation between dosage and duration of action cannot be assumed to be linear or directly proportional.

3.4 Drug Accumulation and Discontinuation

When a drug is administered repeatedly at regular intervals, the drug will *accumulate* in plasma based on how much remains from previous doses at the time the next dose is given. The *accumulation ratio* is defined as the area under the plasma concentration curve (AUC) over a dose interval at steady-state divided by the AUC over the same interval after the first dose [92]. The numerical value of the accumulation ratio depends on the relation of the drug's elimination half to the interval between doses (which, in the case of hypnotics, is 24 h) (Fig. 9). If the half-life is much shorter than 24 h, there is little accumulation. Conversely, if the half-life is longer than 24 h, accumulation will occur, since previous doses are not fully eliminated when the next dose is given. Accumulation is a clinical concern mainly for the older BZ agonist hypnotics (nitrazepam, flurazepam, and quazepam). The more recent generation of hypnotics has much shorter half-life values, and accumulation is not an issue (Fig. 10).

Accumulation (or nonaccumulation) of hypnotics during extended therapy has benefits and disadvantages. Long half-life hypnotics are likely to produce residual sedative effects that may increase with repeated doses [93–98]. This seems to be of particular concern for the elderly. However, the degree of cumulative sedation is not directly proportional to the rising plasma concentrations over time, since sedative and performance-impairing effects are partially offset by tolerance. An advantage of long half-life hypnotics is evident at the time of drug discontinuation,

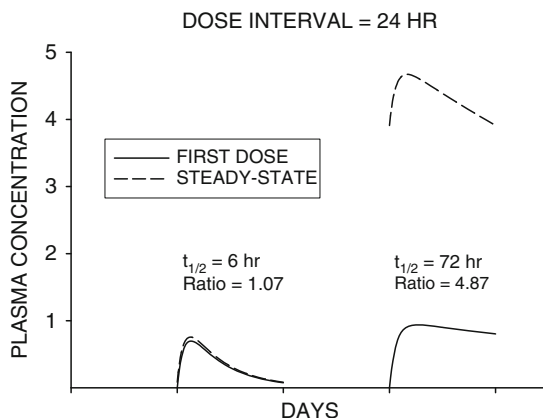


Fig. 9 Hypothetical accumulation of two hypnotic drugs given nightly (dose interval of 24 h) on a repeated basis. The two drugs have different half-life values: 6 h (*left*) or 72 h (*right*). Plasma concentration curves are shown after the first dose (*solid line*), and after nightly dosing such that steady-state has been reached (*dashed line*). The accumulation ratio is the area under the plasma concentration curve (AUC) during a 24-h dosage interval at steady-state divided by the AUC over 24 h after the first dose

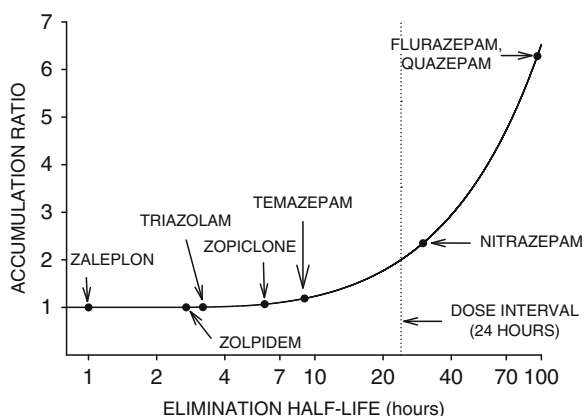


Fig. 10 Typical elimination half-life values for benzodiazepine agonist hypnotics discussed in the text were used to calculate the predicted accumulation ratios (y-axis). The *vertical dotted line* is the dosage interval (24 h). When the half-life is substantially less than the dosage interval, little or no accumulation is predicted. However, when the half-life exceeds the dosage interval, there may be substantial accumulation. The highest accumulation ratio is predicted for flurazepam and quazepam, both of which are precursors of desalkylflurazepam

after which plasma concentrations decline slowly over days or weeks. This “self-tapering” property serves to minimize clinical discontinuation symptoms. For short half-life hypnotics, there is little or no accumulation with repeated dosage, and minimal risk of daytime sedation or cumulative sedative effects. However, rebound

and discontinuation symptoms are of concern when treatment is abruptly stopped [93, 94, 97, 98], and it is strongly recommended that short half-life hypnotics be tapered rather than abruptly stopped when the treatment period is complete.

4 **Pharmacokinetics of Benzodiazepine Agonist Hypnotics**
 (Table 3)

4.1 *Flurazepam and Quazepam: Precursors of*
 Desalkylflurazepam

Flurazepam was the first BZ hypnotic introduced in the United States [35, 43, 44]. At the time of development (the 1960s), the impact of metabolic pathway and elimination half-life were not fully understood. Flurazepam turned out to have a complex metabolic pathway [31, 99–102]. After oral dosage, flurazepam itself – and two other metabolic intermediates – appears in plasma only transiently and at relatively low concentrations (Fig. 11). The principal metabolite is desalkylflurazepam (DAFLZ), which appears to account for pharmacologic activity as determined by experimental neuroreceptor and brain uptake studies [102]. DAFLZ has a very long half-life, leading to extensive accumulation with multiple dosage [101, 103]. Half-life is prolonged and accumulation is more extensive in the elderly, and there is evidence to indicate that the higher dosage levels of flurazepam should be avoided in the geriatric population [104]. Nonetheless, flurazepam was widely prescribed during the 1970s and 1980s, and overall had a favorable safety record.

Table 3 Pharmacokinetic properties of representative hypnotic drugs acting via the GABA-benzodiazepine receptor complex

Parent drug	Principal active metabolites	Half-life range (h)
(Flurazepam)	Desalkylflurazepam	70–110
(Quazepam)	Desalkylflurazepam	70–110
Nitrazepam	–	20–40
Temazepam	–	8–15
Triazolam	–	2–5
Zolpidem	–	2–5
Zaleplon	–	0.5–2
Zopiclone	–	4–7
Eszopiclone	–	5–8

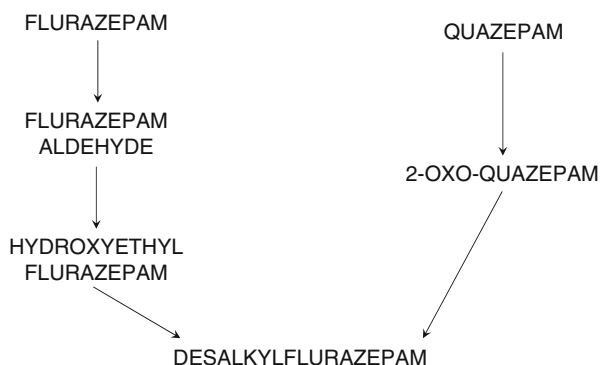


Fig. 11 Schematic metabolic profiles for flurazepam and quazepam, both of which have the same final metabolite, desalkylflurazepam

The metabolic profile of quazepam [105, 106] resembles that of flurazepam. Quazepam and another intermediate metabolite appear transiently, and the final metabolite is DAFLZ (Fig. 11) [107–110].

4.2 Nitrazepam

Nitrazepam is the prototype BZ hypnotic, available in the UK and Europe since the 1960s but never marketed in the US. Nitrazepam is biotransformed to inactive metabolic products via reduction of the nitro substituent. The half-life of nitrazepam falls in the range of 20–40 h [111–114], and produces a degree of accumulation that is moderate, but not as extensive as with DAFLZ. Elimination half-life of nitrazepam is only slightly prolonged in the elderly [114], but dosage nonetheless should be reduced for older patients [115].

4.3 Temazepam

Temazepam is a 3-hydroxy BZ derivative having a half-life in the range of 8–15 h in the majority of individuals [31, 116, 117]. As such, there is some likelihood of residual daytime effects, and a small but detectable degree of accumulation with multiple dosage [118]. An apparent advantage of temazepam is that its metabolic pathway mainly involves glucuronide conjugation as opposed to biotransformation by the Cytochromes P450 [115, 119]. A consequence is that elimination of temazepam is not significantly altered by old age or liver disease [120, 121], and the drug is relatively nonsusceptible to changes in metabolism due to drug interactions

[122–124]. Temazepam has been extensively prescribed in the United States and Europe, and has a favorable safety record.

4.4 Triazolam

Triazolam was the first of the “very short half-life” BZ hypnotics to become available in the United States in the early 1980s. With a half-life in the range of 2–5 h [31, 125], there is essentially no accumulation with repeated dosage, and no risk of residual daytime sedation as long as doses remain within the recommended range. There is consistent evidence of rebound insomnia when the drug is discontinued after multiple-dose treatment [93, 94, 97, 98]. This problem can be minimized or eliminated by tapering the dosage at the end of the treatment period [126].

Triazolam elimination is almost exclusively dependent on Cytochrome P450-3A (CYP3A) enzymes present in the liver and gastrointestinal tract mucosa [127], and in fact it is often used as an index or probe compound to profile CYP3A activity in clinical studies [128, 129]. Oral triazolam has absolute bioavailability (systemic availability) of only 40–50% due to presystemic extraction (first-pass metabolism) by enteric and hepatic CYP3A enzymes [130]. As such, the plasma level profile of triazolam is highly susceptible to changes caused by drug interactions involving CYP3A. Triazolam plasma levels are greatly increased by CYP3A inhibitors such as ketoconazole, itraconazole, ritonavir, and macrolide antibiotics [127, 131–135], and greatly reduced by inducers such as rifampin [136]. Plasma levels are also increased in the elderly, evident mainly as a higher AUC but not necessarily a prolonged half-life [137–139]. Consequently, there is a need for reduced doses to treat insomnia in the geriatric population.

Triazolam nonetheless has an overall favorable safety record, and is a reasonable therapeutic option as a short half-life BZ hypnotic.

4.5 Zolpidem

Though not a BZ in structure, zolpidem nonetheless acts via the GABA-BZ receptor system. Zolpidem has a half-life in the range of 2–5 h [140–144], and in many ways it is similar to triazolam in terms of systemic pharmacokinetics and clinical BZ agonist effects [144–149]. However, a potential benefit of zolpidem is that its metabolic clearance is not solely dependent on CYP3A – a number of other Cytochrome P450 enzymes also contribute to biotransformation of zolpidem [150]. A consequence is that metabolic inhibitors of CYP3A have only a small or modest effect on plasma levels of zolpidem [135, 151–154]. Nonetheless, plasma zolpidem concentrations are substantially increased in the elderly [155] and doses should be reduced in the geriatric population.

In the United States, the conventional immediate-release (IR) zolpidem tablet is available in generic form at relatively low cost, and is the single most widely prescribed hypnotic agent (Table 2). Modified- or controlled-release (CR) zolpidem has recently become available for prescription, though only as a brand name (Ambien-CR) at much higher cost. Controlled-release zolpidem is actually a dual-release preparation: one component is immediate-release, and the second compartment is slow-release for the purpose of sustaining effective plasma concentrations during the middle of the sleep cycle [156, 157]. Clinical kinetic-dynamic studies do in fact show that CR zolpidem achieves this, though the effect is subtle [158]. An unanswered question is the extent to which kinetic and dynamic differences between the IR and CR zolpidem are explained simply by differences in net dosage (10 mg for IR versus 12.5 mg for CR) as opposed to the dual release mechanism.

4.6 *Zaleplon*

Zaleplon has a very short half-life of approximately 1–1.5 h in most subjects [143, 159]. Consequently, there is no accumulation, and in fact favorable drug effects in terms of prolonging sleep duration are difficult or impossible to demonstrate [80–83]. This may explain why zaleplon has never achieved extensive use in clinical practice. A number of pharmaceutical companies are working on development of formulations to sustain the action of zaleplon through delayed or prolonged release mechanisms. None of these products had become clinically available by the end of 2009.

4.7 *Zopiclone and Eszopiclone*

Racemic zopiclone has been available as a hypnotic outside the United States for many years, but only the S-enantiomer (eszopiclone) is marketed in the US [77–79].

The half-life of racemic zopiclone falls in the range of 4–8 h [160, 161]. While accumulation is minimal, there is concern regarding residual drug effects due to plasma levels that are sustained beyond 8 h. Clearance of zopiclone depends mainly on CYP3A [162], and plasma levels are increased by erythromycin and reduced by rifampin. Zopiclone clearance is reduced and plasma levels increased in the elderly [160]. There is essentially no published data on the pharmacokinetics of eszopiclone – available information is limited to what is printed in the product label. According to that source, the kinetic properties of eszopiclone resemble those of the racemic mixture. However, the clearance of S-zopiclone is lower, and the half-life longer, than those of R-zopiclone or the racemic mixture.

5 Comment

Drugs acting via the GABA-BZ receptor complex continue to be the principal option available for the pharmacologic management of insomnia. No single drug fully achieves the therapeutic objective of shortening sleep latency, prolonging sleep duration, and reducing nocturnal awakenings with minimal or no risk of excessively prolonged sedation or hangover. The intrinsic constraints of human physiology and pharmacokinetics make it unlikely that any single pharmacologic entity will ever fully achieve the goal. Novel approaches to drug delivery may ultimately allow improvements in drug efficacy and safety – particularly devices that can regulate the delivery and exposure to short half-life hypnotics. So far, drugs acting by mechanisms other than GABA-BZ agonism – such as the melatonin receptor agonists – have not emerged as major therapeutic breakthroughs.

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Part II

Sleep Science and Circuitry

Sleep and Its Modulation by Substances That Affect GABA_A Receptor Function

Axel Steiger

Abstract The GABA_A receptor is the target of various endogenous and synthetic substances modulating sleep–wake behavior. According to its physiological role, the GABA_A receptor mediates not only the effects of sleep-promoting substances but also of the whole range of agonists, partial agonists, antagonists, partial inverse agonists, and inverse agonists, resulting in a wide spread spectrum of the effects on sleep ranging from sleep promotion to sleep inhibition. Synthetic agonists at the GABA_A receptor include barbiturates, which are obsolete today, the classical benzodiazepines and the so-called nonbenzodiazepine hypnotics. Generally, these substances help to enter and to maintain sleep. Their effects on sleep architecture and microstructure of sleep are opposite to those of sleep promotion after sleep deprivation as these agonists suppress slow wave sleep, rapid-eye-movement (REM) sleep, and electroencephalogram (EEG) power in the lower frequency bands, whereas they enhance EEG power in the higher frequency bands. In contrast, the selective extrasynaptic GABA_A receptor agonist gaboxadol and the GABA-uptake inhibitor tiagabine mimic the effects of sleep deprivation. The synthetic partial agonist bretazenil shares some of the sleep-EEG effects of benzodiazepines. The synthetic antagonist flumazenil exerts some effects on sleep EEG, suggesting that the substance has its own intrinsic effects or acts as an antagonist to an endogenous ligand. A few previous studies report inverse agonistic effects of β -carbolines on sleep-EEG recordings. Furthermore, certain neuroactive steroids (pregnenolone, THDOC, progesterone, allopregnanolone, dehydroepiandrosterone) and neuropeptides (growth hormone-releasing hormone, neuropeptide Y, and galanin) exert specific effects on sleep EEG, which may be mediated by the GABA_A receptor. This chapter aims to review the state of the art in this field.

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1 Introduction

The GABA_A receptor is the target of various endogenous and synthetic substances modulating sleep–wake behavior. These include the benzodiazepines and the so-called nonbenzodiazepine hypnotics, together the today world-wide most frequently used type of hypnotics. According to its unique physiological role, the GABA_A receptor mediates not only the effects of sleep-promoting substances but also of the whole range of agonists, partial agonists, antagonists, partial inverse agonists, and inverse agonists [1]. Correspondingly, a wide spread spectrum of effects on sleep are induced by these substances ranging from sleep promotion to sleep inhibition. The endogenous substances modulating sleep via the GABA_A receptor include neuroactive steroids and neuropeptides.

The effects of these substances on sleep–wake behavior are assessed by sleep electroencephalogram (EEG). Mammalian sleep consists of periods of rapid-eye-movement (REM) and non-REM sleep. In healthy young subjects, non-REM- and REM-sleep periods occur alternating in a cyclic fashion. Non-REM sleep consists of four stages according to the criteria by Rechtschaffen and Kales [2]. A recent classification [3] differentiates between three stages of non-REM sleep only. During sleep stage 1 (or N1, respectively), a slowing of EEG activity occurs. Stage 1 is the transition from drowsiness to light sleep. Characteristic elements of stage 2 (N2) sleep are sleep spindles and K-complex wave forms. Slow wave sleep (SWS), the sleep stages 3 and 4 [2] or N3 [3], is characterized by synchronized slow waves. Faster EEG activity, horizontal rapid eye movements, and hypotonus of skeletal muscles are found during REM sleep. In animals, particularly in most species that are used for experiments, sleep EEGs resemble those in humans [4], whereas in contrast to human sleep, animal sleep is characteristically polyphasic with episodic sleep–wake patterns. Animal non-REM sleep is not divided into stages, whereas its depth can be interpreted according to EEG power [5]. Since recording of rapid eye movements is difficult in small animals, their REM sleep criteria include combination of wake-like EEG with flat electromyogram indicating muscle atonia. The microstructure of sleep is examined in humans and animals as well by computerized quantitative EEG analysis of amplitudes and power in the EEG bands β , σ , α , τ , and δ . In a young healthy human subject shortly after going to bed, sleep stages 1, 2, and SWS occur consecutively. The mean duration of the first non-REM period lasts 20 min. During this interval, the major amount of SWS and of slow wave activity (SWA, EEG δ power) occurs. In most subjects, 4–5 sleep cycles occur during the night. Each cycle consists of one episode of non-REM sleep and one of REM sleep. By contrast, in animals, one non-REM episode lasts a few seconds to a few minutes. Short periods of wakefulness or REM sleep often interrupt the non-REM episodes. In humans, the first half of the night is dominated by SWS, whereas during the second half of the night, non-REM stage 2 and REM sleep preponderate. Similarly in rats and mice during the light period, their resting phase, first the major portion of non-REM sleep occurs, which is followed later on by REM sleep.

Various synthetic and endogenous ligands of the GABA_A receptor modulate this sleep pattern in a different fashion. This chapter aims to present the state of the art of clinical and preclinical research in this field.

2 Synthetic Agonists

2.1 *Barbiturates*

Barbiturates are the oldest class of hypnotics acting at the GABA_A receptor. Nowadays, these drugs are obsolete due to their toxicity. The effect of barbiturates on GABA_A receptor functions appears to be dose-dependent. High (anesthetic) concentrations are capable of opening GABA_A-associated chloride channels [6]. Lower concentrations of barbiturates increase the affinity for and enhance the response to GABA by increasing the average open duration of the chloride channels [7]. In rats, pentobarbital shortens sleep latency, increases preREM sleep, a transitional state between non-REM and REM sleep, and decreases REM sleep [8, 9]. In healthy volunteers, phenobarbital and thiopental decrease wakefulness and increase SWS and REM latency [10, 11]. Generally, barbiturates increase the ability to enter and to maintain sleep, increase non-REM sleep and the occurrence of sleep spindles, and decrease REM sleep (review: [9]).

2.2 *Benzodiazepines*

In the absence of GABA, benzodiazepines are inactive at the GABA_A receptor but enhance the frequency of GABA-induced channel openings [12]. In rats, after acute systemic administration of benzodiazepines such as flurazepam, diazepam, triazolam, or midazolam, sleep latency decreases dose-dependently and increases non-REM sleep and pre-REM sleep. The latter effect is most distinct during the dark period, when the amount of sleep is low in these night-active animals. Furthermore, after these drugs, REM latency increases and REM time decreases (review: [9]). In EEG spectral analysis, a decrease of slow EEG components and an elevation of the spindle and higher frequency range occur within non-REM sleep in a dose-dependent manner (see Fig. 1) [13, 14]. In healthy volunteers, benzodiazepines shorten sleep latency, increase the total sleep time, reduce the number of awakenings and body movements, and enhance distinctly time spent in stage 2, whereas stage 1, SWS and REM sleep are reduced (see Fig. 2). The latter effect is related to an increase of REM latency and a decrease of the number of REM episodes [15]. Similar to the findings in the rat, in humans, benzodiazepines decrease EEG power density in the lower frequencies and elevate activity in the spindle frequency range during non-REM sleep [16]. A rapid development of tolerance to hypnotic action

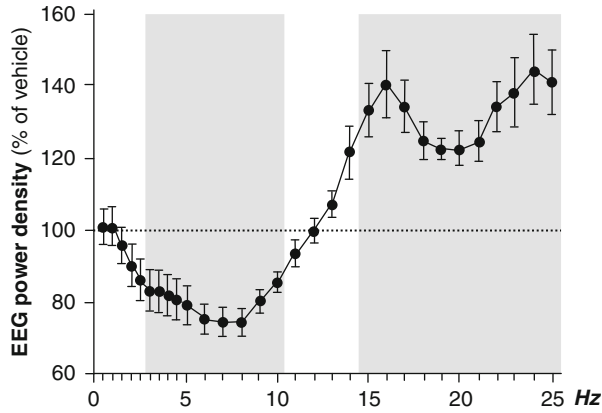


Fig. 1 EEG power densities within non-REM sleep over an 8-h recording period after 3 mg/kg midazolam in rats ($n = 8$). For each animal and each frequency band, average power densities were computed, normalized, and then expressed in percent of the average power densities of the corresponding frequency and vigilance state of vehicle. *Shaded areas* indicate the frequencies for which ANOVA revealed a significant effect on the factor condition. From Lancel et al. [14]. Reprinted by permission from Macmillan Publishers Ltd: Neuropsychopharmacology, copyright 1996

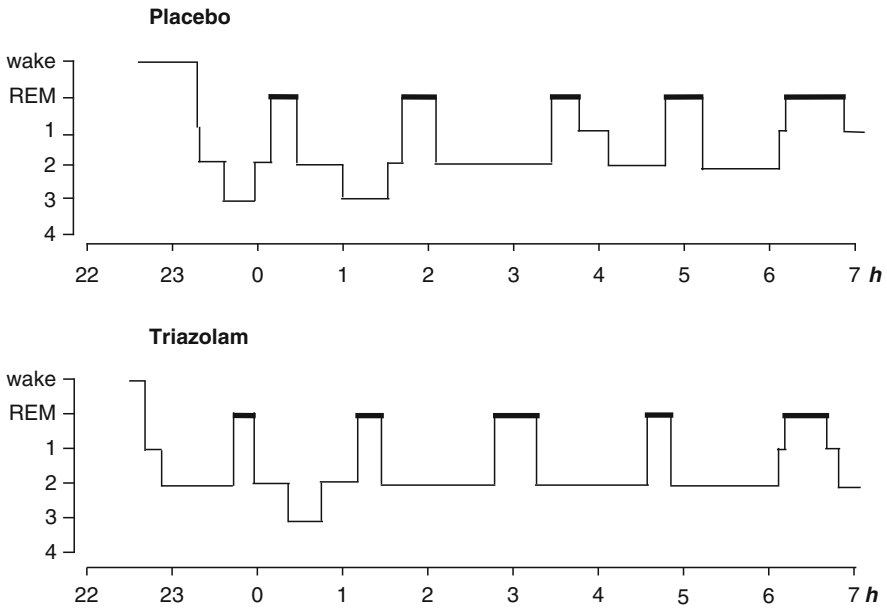


Fig. 2 Hypnograms of a young healthy volunteer after placebo and after 0.25 mg triazolam. REM – rapid eye movement sleep, 1–4 indicate stages of non-REM sleep

occurs with benzodiazepines. Whereas after acute administration of benzodiazepines a marked sedation is observed as evident by reduced locomotor activity and exploratory behavior in rodents, already after 3 days of continuous dosing, the benzodiazepines, lorazepam, and triazolam did not reduce locomotion and exploration in rats [17]. The sleep-promoting action of triazolam vanished within 5 days of continuous drug administration [18]. Accordingly, in humans, the somnogenic effects of benzodiazepines are lost after a few days to weeks of chronic administration [19]. One study compared sleep EEG in three groups of adults aged 55 years or older: patients with insomnia using benzodiazepines chronically, drugfree insomnia sufferers, and self-defined good sleepers. No difference was found between drug-free insomnia sufferers and good sleepers. Patients with insomnia using benzodiazepines spent more time in sleep stage 2 and less time in SWS than the good sleepers. A higher count of microarousals was observed in the patients using benzodiazepines compared to the other two groups. Benzodiazepine users showed significantly less δ - and τ -activity over the night than the good sleepers. In comparison to drugfree insomnia sufferers, the benzodiazepine users had less δ - and τ -activity in sleep cycle 2. The benzodiazepine users showed more β -1 activity within sleep cycle 3 than good sleepers. Furthermore, β -1 activity was higher in the benzodiazepine users than in the other groups within sleep cycle 4 [20].

According to their pharmacokinetic profile, the benzodiazepine hypnotics available today can be divided into three categories:

1. Substances with long elimination half-life (40–250 h): flurazepam, quazepam. The elimination half-life of the prodrug flurazepam is short (1–2 h), whereas it has two active metabolites, hydroxyethylflurazepam (elimination half-life ca. 1–3 h) and desalcyflurazepam (elimination half-life 40–250 h).
2. Substances with intermediate elimination half-life (8–40 h): estazolam, flunitrazepam, loprazolam, lormetazepam, nitrazepam, and timazepam. The elimination half-life of flunitrazepam is 10–30 h, of its active metabolite desmethylflunitrazepam is 20–30 h. Nitrazepam has no active metabolite. The metabolites of loprazolam, lormetazepam, and timazepam are of little pharmacological relevance.
3. Substances with short elimination half-life (4–8 h): brotizolam and midazolam. The metabolite of brotizolam is of little pharmacological relevance.
4. Substances with ultrashort elimination half-life (1–5 h): triazolam. Its metabolite is without pharmacological relevance [21, 22].

Not all substances are available in all countries.

Side effects of benzodiazepines are headaches, confusion, ataxia, dysarthria, blurred vision, gastrointestinal disturbances, paradoxical excitement, and anterograde amnesia, whereas their incidence is low and these drugs are generally well tolerated. Treatment with benzodiazepines has been associated with at least 50% increase in the risk of hip fracture, particularly in elderly patients. When benzodiazepine treatment is discontinued, withdrawal symptoms may occur. These symptoms are not found before benzodiazepine treatment and should be differentiated from rebound phenomena, e.g., enhanced recurrence of the original symptoms,

mainly anxiety or insomnia. Incidence and degree of withdrawal symptoms and of rebound phenomena depend on the duration and dosage of treatment (being lower for intermittent or variable dosages) and on various personality factors. In order to lower the risk and the intensity of withdrawal and rebound symptoms, a cessation of benzodiazepines should be performed by gradual dose reduction [23]. One study investigated the sleep EEG of patients with insomnia during continuous and very long lasting use of benzodiazepines and after withdrawal of these substances. A group of 25 patients who had been taking benzodiazepines every night for 6.8 ± 5.4 years was compared to 18 age-matched healthy controls. Sleep EEG was recorded during night 1 while taking benzodiazepines, during night 2 (first night after completing benzodiazepine withdrawal) and night 3 (15 days after gradual benzodiazepine withdrawal). At night 1, a significant decrease of total sleep time and increased sleep latency were found in the patients in comparison to controls. An increase in stage 2 and a decrease in SWS were observed in comparison to night 3. EEG activity in the σ -band was increased and δ -count in stages 2–4 of non-REM sleep and REM sleep was decreased in the patients in comparison to night 3. During benzodiazepine withdrawal, 6 of 9 patients taking lorazepam failed withdrawal. In the remaining 19 subjects, a gradual cessation of benzodiazepines was associated to immediate worsening of sleep. At night 3, however, some of the sleep-EEG variables in patients did not differ from night 1, while taking benzodiazepines, except for a significant increase in SWS and in δ -count and a decrease in stage 2 sleep. These values did not differ from those of control subjects. REM-sleep variables did not differ across conditions. Subjective sleep quality was improved at night 3 compared to night 1. The authors concluded that chronic intake of benzodiazepines may be associated with poor sleep. A progressive withdrawal during 15 days did not avoid an immediate worsening of sleep-EEG variables. However, at the end of the protocol, SWS, δ -count, and sleep quality were improved compared to the time of chronic benzodiazepine intake [24].

The risk of developing dependency is of concern. Benzodiazepine dependency is developed quickly after excessive dosages and after longer duration of treatment. It is thought that approximately 10–30% of long-term users develop dependency [25].

Benzodiazepines are relatively safe and their toxicity is minimal. Tiredness and drowsiness (reduced concentration and impaired psychomotor function) may occur, particularly at the beginning of treatment, and that leads to accidents. Dizziness, numbness, incoordination, and ataxia are signs of relative overdosages [23]. Generally, hypnotics including benzodiazepines should not be prescribed for periods exceeding 4 weeks. In patients with intermittent insomnia, hypnotics may be prescribed during 4–6 nights per month. Administration should start at low dosages [22].

2.3 Nonbenzodiazepine Hypnotics

The so-called nonbenzodiazepine hypnotics bind to the benzodiazepine site but share some selectivity for the α -1-receptor subtype compared to the classical

benzodiazepines. Clear generalizations about clinically important differences between these two classes cannot be made [26]. The term “nonbenzodiazepine hypnotics” refers particularly to the chemical structure of the substances and less to the mechanism of action. Benzodiazepines and nonbenzodiazepine hypnotics are antagonized by flumazenil [22]. A meta-analysis suggests that nonbenzodiazepines are safer than benzodiazepines [27]. The risk for tolerance and addiction appears to be lower after nonbenzodiazepines than after benzodiazepines. However, it should be kept in mind that this risk exists also for nonbenzodiazepines [22, 28].

2.3.1 Zolpidem

The imidazopyridine derivative zolpidem is short-acting at the α -1 receptor with intermediate potency at the α -2 and α -3 receptors [29]. In rats, zolpidem shortens sleep latency, increases time spent in non-REM sleep, and decreases REM sleep dose-dependently as a function of dose. The latter effect is associated with a prolonged appearance of the first REM episode and with a transient reduction of the number of REM episodes. PreREM sleep remains widely unchanged. Promotion of non-REM sleep and the suppression of REM sleep occur both during the light and dark periods (review: [9]). In young healthy volunteers, only weak effects on nocturnal sleep were found (slight increase in total sleep time, shortened sleep onset latency, decrease in REM sleep by trend, slight delay in REM latency) (review: [9]). Zolpidem exerts only minimal effects on the time spent in each of the non-REM-sleep stages. EEG spectral analysis within non-REM sleep shows a distinct decrease of power in the low frequency bands and an enhancement in the range of sleep spindles, resembling the effects of classical benzodiazepines [30]. More distinct effects of the substance on sleep were found during daytime when sleep propensity is relatively low. During this condition, it appears to increase total sleep time, mainly due to a decrease in the number of awakenings and an increase in stage 3, whereas REM sleep decreases [31].

In patients with insomnia, zolpidem decreases sleep latency and nocturnal awakenings and increases total sleep time. Therefore, the substance is thought to be appropriate for patients with disorders of maintaining sleep [32]. Whereas zolpidem is indicated for short-term use, some studies suggest that it can be applied for longer treatment periods. In a placebo-controlled study, improved sleep latency and sleep efficiency were reported throughout 35 days [33]. The efficacy of a dose of 10 mg zolpidem taken intermittently (3–4 nights weekly) persisted during 3 months as indicated by improved sleep latency, nocturnal awakenings, and total sleep time [34]. Further chronic studies suggest that clinical doses of zolpidem are not associated with the occurrence of tolerance, or that this risk is marginal only [35]. Some studies reported a rebound insomnia after withdrawal of the substance (review: [26]). There exist some case reports on dependence after prolonged use of zolpidem in patients with a history of substance abuse [36]. The rates of abuse and dependency for zolpidem are reported to be similar to those of some benzodiazepines [37].

An extended-release preparation of zolpidem was approved in the US for patients with impaired sleep maintenance. The tablets permit a certain portion of the drug to be released immediately, whereas the remaining content is released slower, resulting in steady plasma concentrations during the middle of the night [38].

2.3.2 Zopiclone, Eszopiclone

The binding spectrum of the cyclopyrrolone zopiclone resembles that of the classic benzodiazepines [39]. After administration to rats during the light period, zopiclone increases wakefulness by shortening sleep latency and increases non-REM sleep and decreases preREM and REM sleep [40]. The substance exerts a weak effect on conventional sleep-EEG variables in healthy volunteers (decrease of stage 1, increase of stage 2 and of REM latency) [41]. During a phase-advanced sleep schedule, the microstructure of sleep was changed distinctly after zopiclone and after midazolam as well as EEG power density in the lower frequency range was reduced and the activity in the spindle-frequency range were increased, resembling again the effects of classical benzodiazepines [42]. In patients with insomnia, zopiclone decreases sleep latency and the number of nocturnal awakenings and increases sleep duration [43]. Next day impairment after 7.5 mg zopiclone but not 10 mg zolpidem in a driving-simulator test performed 9–11 h after dosing was reported [44]. The incidence of withdrawal symptoms was reported to be considerably lower after zopiclone than after classical benzodiazepines. Zopiclone dependency was reported predominantly after high dosages in patients with a history of drug abuse [45].

Eszopiclone is the pharmacologically active S-isomer of zopiclone. It was approved for treatment of sleep onset latency and sleep maintenance insomnia with no short-term restrictions in the US [46]. The Committee for Medicinal Products for Human Use of the European Medicines Agency had given a positive opinion on escopiclone. However, the Committee has also concluded that eszopiclone could not be considered to be a new active substance [47].

2.3.3 Zaleplone

The pyrazolopyridine zaleplone exerts greater potency at the α -1, β -2, δ -2 receptor subtypes than at α -2- and α -3-containing receptors [48]. Zaleplone is rapidly absorbed and has an elimination half-life of approx. 1 h [32]. Zaleplone significantly reduces sleep latency. Its effects on sleep maintenance are less consistent than those of zolpidem probably related to its shorter half-life [49]. Accordingly, zaleplone is indicated for the treatment of patients with difficulties falling asleep. It is possible to give zaleplone up to 4 h before final awakening. Therefore, it may be used in patients who awaken after a short sleep time [50]. In the rat model, the sleep-promoting action of zaleplone disappeared within 5 days of administration [51]. In contrast, after 4–5 weeks of administration, no evidence of

tolerance or rebound insomnia was found in patients with primary insomnia and with insomnia-related psychiatric disorders [52, 53]. However, it was suggested that the abuse potential of zaleplone is similar to that of triazolam and zolpidem [37].

2.3.4 Indiplon

The pyrazolopyrimidine indiplon is structurally similar to zaleplone [54]. It has a high affinity for α -1-containing GABA_A receptors [55]. The substance was developed in two different formulations for two different types of insomnia: indiplon-immediate release (IR) was developed for sleep-onset problems, whereas indiplon-modified release (MR) was designed for impaired sleep maintenance. Three months nightly treatment of adults with primary insomnia resulted in a significant improvement relative to placebo (subjectively less latency to sleep onset, sleep maintenance, and sleep quality) [56]. In another study in patients with chronic insomnia, indiplon was used as “as-needed” strategy in response to difficulties falling back to sleep after middle of the night awakening. Latency to sleep onset after such event is reduced significantly compared to placebo [57]. Whereas there are only a few peer-reviewed articles on indiplon clinical trial results, the information available so far suggests that the substance is well tolerated and improves subjective and objective sleep variables in adults and elderly patients with insomnia [58]. No data on the effects of indiplon on sleep EEG in laboratory animals or on microstructure of sleep exist. Indiplon is not marketed so far.

2.4 *Selective Extrasynaptic GABA_A Receptor Agonist*

2.4.1 Gaboxadol

Gaboxadol (4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridine-3-ol) (THIP) is a selective extrasynaptic GABA_A receptor agonist (SEGA). It activates GABA_A receptors, which are generally located in regions outside of the synapse mediating tonic inhibition. Gaboxadol binds at the interface between α and β subunits, the binding site of GABA [26]. The pattern of sleep-EEG effects of gaboxadol differs distinctly from that of benzodiazepines and nonbenzodiazepine hypnotics. Non-REM sleep and SWA increase after intraperitoneal administration of gaboxadol in rats at the beginning of the light period, whereas REM sleep remains unchanged [59]. To investigate the effects of repeated administration of gaboxadol, sleep EEG was recorded in rats before, during, and after 5 days of intraperitoneal administration of the substance in comparison to placebo. At baseline, the gaboxadol and the placebo group showed similar sleep patterns. After the first gaboxadol injection, more non-REM sleep, longer non-REM sleep episodes, and higher levels of SWA in non-REM sleep were found than after placebo. These effects persisted during all treatment days. REM sleep remained unaffected after drug cessation. The sleep

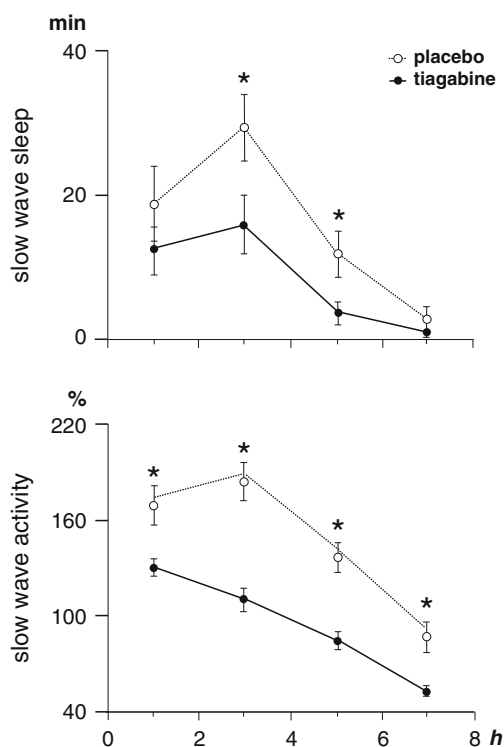
patterns of the gaboxadol and the placebo group were practically identical again. These findings suggest that gaboxadol does not rapidly produce tolerance regarding its sleep-EEG effects. Abrupt withdrawals appear not to be associated with sleep disturbances [60]. Also in young male healthy volunteers, gaboxadol increases SWS and SWA. It reduces EEG activity in the spindle frequency range and does not affect REM sleep [61]. Furthermore, gaboxadol improves disturbed sleep in healthy elderly subjects [62, 63] and during postnap sleep in healthy volunteers [64]. In these conditions, after gaboxadol, intermittent wakefulness decreases whereas total sleep time, SWS, and SWA increases. In healthy elderly subjects, attention and memory function were not influenced by gaboxadol [63]. Behavioral, psychological, and physiological measures of the impact of sleep restriction were assessed in two groups of healthy volunteers who received either gaboxadol or placebo. Both groups underwent four nights of sleep restriction to 5 h. The placebo group displayed the predicted deficits due to sleep restriction. In contrast, after gaboxadol, less sleepiness and fatigue were found. The study results support the view that enhanced SWS, in this study after gaboxadol, reduces physiological sleep tendency, sleepiness, and fatigue typically resulting from sleep restriction [65]. A 2-week efficacy and safety study of gaboxadol and zolpidem using electronic diaries was performed in outpatients with primary insomnia. A dosage of 15 mg gaboxadol improved sleep onset and maintenance variables, sleep quality, and day time function as did zolpidem. After discontinuation of the short-time treatment with gaboxadol, withdrawal symptoms or rebound insomnia occurred, whereas for zolpidem, transient rebound insomnia was reported [66]. Two randomized, placebo-controlled, 30-night sleep-EEG studies on the effects of gaboxadol on sleep in adult and elderly patients with primary insomnia showed that gaboxadol enhanced sleep maintenance and SWS but had little or no effect on sleep onset [67]. The clinical development of gaboxadol for the treatment of insomnia was stopped based on an assessment of its overall clinical profile in phase III trials, including limited or variable efficacy and the occurrence of psychiatric side effects at supratherapeutic doses in an abuse liability study involving drug abusers [68].

2.5 GABA-Uptake Inhibitor

2.5.1 Tiagabine

The GABA-uptake inhibitor tiagabine is in clinical use as anticonvulsant. After intraperitoneal administration of tiagabine in rats, EEG power density at all frequency bands during non-REM sleep is elevated dose-dependently, most prominent in the lower frequencies. REM sleep is slightly suppressed [69]. After a single oral dose of tiagabine in healthy elderly subjects, sleep efficiency, SWS, and SWA increase and wakefulness decreases by trend [70] (see Fig. 3). In another sample of elderly subjects, the sleep-promoting effect of tiagabine was confirmed [71].

Fig. 3 Time course of slow wave sleep (*upper graph*) and slow wave activity in the EEG within non-REM sleep (*lower graph*) after administration of placebo or tiagabine. Values are mean \pm SEM ($n = 10$) and are plotted in the middle of the 2-h intervals. For each subject, the slow wave activity data were expressed as percentage of the average slow wave activity within non-REM sleep during the entire placebo night. There were significant differences in SWS and SWA between the treatments (repeated measures ANOVA). * $P < 0.05$ (two-sided paired t tests). From Mathias et al. [70], with permission from Elsevier



3 Synthetic Partial Agonist

3.1 Bretazenil

The imidazodiazepinone derivate bretazenil exhibits potent anxiolytic and anticonvulsant effects in animals. In contrast to full benzodiazepine agonists, however, it causes only minor motor impairment and mild sedation [72, 73]. In vitro studies with transfected cell lines suggest that the different functional effects of bretazenil and diazepam are not due to binding in different GABA_A receptor subunits but are caused by a less efficient modulation of the same receptor sites [74]. In clinical trials, the efficacy of the substance as anxiolytic drug was tested. The substance was never marketed. In healthy male volunteers, the effects of 1 mg bretazenil on sleep EEG were examined. With regards to sleep architecture, the sleep EEG shared some but not all the effects of benzodiazepine agonists on sleep. Sleep continuity variables (sleep period time, sleep latency) remained unchanged after the substance. Sleep stage 2 increased. SWS was decreased only during the last third of the night but not during the total night. Similarly, the amount of REM sleep was reduced only during the second third of the night. Furthermore, REM latency increased. Spectral analysis of sleep-EEG power showed a decrease in δ and τ power and a tendency to

increased σ power activity after bretazenil. In all, bretazenil failed to show the whole spectrum of full benzodiazepine agonistic effects on the sleep EEG [75].

4 Synthetic Antagonist

4.1 Flumazenil

Previously, it was claimed that flumazenil is a pure receptor-blocking agent, devoid of any intrinsic activity [76]. By contrast, however, agonistic as well as inverse agonistic effects of this substance on behavior and electrophysiological variables were reported [77, 78]. In healthy volunteers, a prolongation of sleep latency was found after flumazenil [79]. In ten healthy male volunteers, the effects of flumazenil alone or in combination with the benzodiazepine agonist midazolam, placebo, and midazolam alone were compared [80]. Compared to placebo, flumazenil alone prompted an increase in sleep latency, a decrease in SWS, and an increase in wakefulness. As expected, sleep latency was shortened after midazolam. For the first third of the night the amount of intermittent time awake was larger after flumazenil than after midazolam. The amount of SWS was significantly smaller after flumazenil than after midazolam. The combination of midazolam and flumazenil did not differ from placebo (see Fig. 4). These results suggest that flumazenil exerts both agonistic, inverse agonistic, and antagonistic effects. A later sleep onset and increased intermittent wakefulness after flumazenil are opposite to the effects

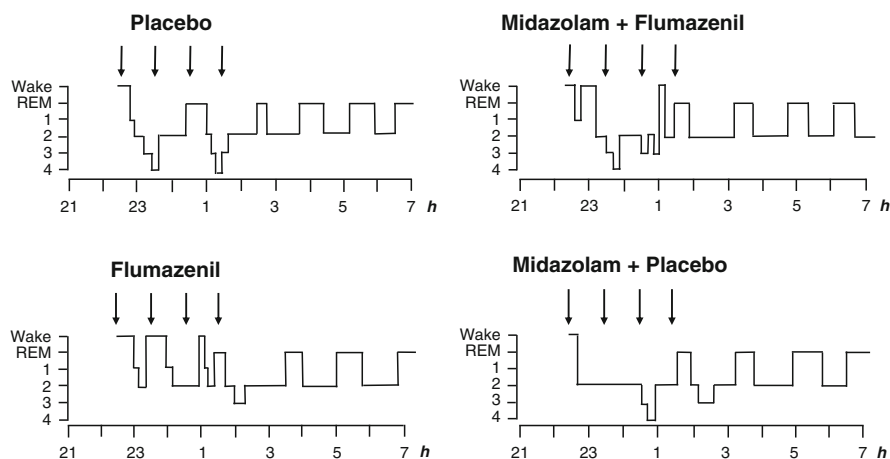


Fig. 4 Hypnograms in a normal control subject after four protocols: (a) $4 \times$ placebo, (b) 1 mg midazolam + $4 \times$ 1 mg flumazenil, (c) $4 \times$ 1 mg flumazenil, (d) 1 mg midazolam + $3 \times$ placebo. Arrows indicate time of application of these substances. REM rapid eye movement sleep, 1–4 – stages of non-REM sleep. From Steiger et al. [80]. With permission of Springer Science+Business Media

of benzodiazepine agonists including midazolam in this study and point to intrinsic inverse agonistic action of flumazenil. Alternatively, flumazenil may antagonize an endogenous GABA_A agonist. In addition to these inverse agonistic or antagonistic effects, flumazenil also appears to exert an agonistic effect, suggested by the decrease in the amount of SWS.

The effects of flumazenil on sleep EEG during early morning recovery sleep (0500–0800 h) following sleep deprivation (2300–0500 h) were investigated in healthy male volunteers. As expected after sleep deprivation, SWS and EEG δ and τ power during non-REM sleep increased and sleep onset latency decreased. Administration of flumazenil during recovery sleep prompted decreases of SWS, δ , and τ power, an increase of stage 2 sleep and a tendency to prolong sleep onset latency [81]. Obviously, flumazenil antagonizes sleep promotion after sleep deprivation. This finding suggests that GABAergic mechanisms contribute to the sleep-EEG changes during recovery sleep. Furthermore, flumazenil showed again antagonistic (prolongation of sleep latency) and agonistic (increase of SWS, δ , and τ power), increase of stage 2 sleep effects. Partial sleep deprivation was performed in patients with major depression. In a double-blind, randomized protocol, either flumazenil or placebo was given during sleep deprivation. The aim was to determine whether flumazenil reduces sleep propensity and thus increases the antidepressant effect of sleep deprivation. EEG was recorded continuously for 60 h for the assessment of microsleep episodes baseline and during sleep deprivation. After flumazenil, the frequency and total amount of microsleep were significantly suppressed, while the antidepressive effect of deprivation was not different between placebo and flumazenil during the deprivation, and the subjective mood improved after the recovery night in patients treated with flumazenil [82]. Flumazenil appears to exert inverse agonistic effects in this study by suppressing microsleep.

5 β -Carbolines

In the 1980s, a series of studies with β -carboline derivatives was performed [83]. Ro15-3505, an imidazobenzodiazepinone was shown to have some inverse agonistic activity. It showed proconvulsant activity in animals. In healthy subjects, it increased vigilance [84].

The synthetic β -carboline derivative ZK 93426 (5-isopropoxy-4-methyl- β -carboline-3-carboxylic acid ethyl ester) exerts anticonvulsant activity in test seizures in animals [85]. However, some proconflict activity and anxiogenic activity in the social interaction test [86, 87] point to inverse agonistic features. Self-rating scales indicate an activating effect at 2 h after intake of the substance [88]. In another study, four groups of ten healthy male volunteers were given either placebo or lormetazepam followed 30 min later by placebo or ZK 93426. Sleep EEG was recorded for 60 min. In addition, multiple sleep latency tests were performed. Within 10 min of injection of lormetazepam, sleep stages 2–4 occurred. Subsequent administration of placebo had no effect on sleep whereas after ZK 93426,

awakening followed after 10–15 min. Subjects who had received placebo only reached sleep stages 1–2 during a 60 min recording. Injection of ZK 93426 prevented subjects from falling asleep suggesting that the substance increases wakefulness. In the multiple sleep latency test, latency to stage 1 was reduced in the lorazepam group. This effect was reversed by ZK 93426. These data suggest that ZK 93426 can antagonize the effects of a benzodiazepine agonist. Some weak inverse agonistic properties of the compound were noted [83].

6 Neuroactive Steroids

Certain steroids, the so-called neuroactive steroids, exert direct effects on neuronal membranes and thereby rapidly affect CNS excitability [89]. Their effect on neuronal excitability is thought to be mediated by the GABA_A receptor. Neuroactive steroids were found to be involved in the regulation of memory, anxiety, and sleep. Glial cells synthesize certain neuroactive steroids independently of peripheral steroid sources [90]. Specific effects on various neuroactive steroids on sleep EEG were identified in humans and rats.

6.1 Pregnenolone

After an oral dose of 1 mg pregnenolone in young male healthy volunteers, SWS increases and EEG power decreases in the spindle frequency range [91] (see Fig. 5). This effect is compatible with a partial inverse agonistic action at the GABA_A receptor. Similarly, subcutaneous administration of pregnenolone at the beginning of the light period in rats results in increased SWA [13]. After intraperitoneal administration of pregnenolone sulfate, REM sleep increased in rats [92]. Also, this effect points to a partial inverse agonistic action of pregnenolone. This view is further supported by the observation that healthy volunteers who were chronically pretreated with high doses of pregnenolone (up to 30 mg) showed less sedation after a single dose of diazepam than subjects pretreated with placebo [93].

6.2 3 α , 5 α -THDOC

3 α , 5 α -tetrahydrodeoxycorticosterone (3 α , 5 α -THDOC), the ring A-reduced metabolite of deoxycorticosterone, occurs in the brain. It is a barbiturate-like ligand of the GABA_A receptor. Sleep EEG was examined in the rat after 3 α , 5 α -THDOC alone and in combination with the benzodiazepine agonist flurazepam. Dose-dependent 3 α , 5 α -THDOC shortened sleep latency and increased non-REM sleep. Similar effects occurred after flurazepam. Furthermore, REM sleep decreased after

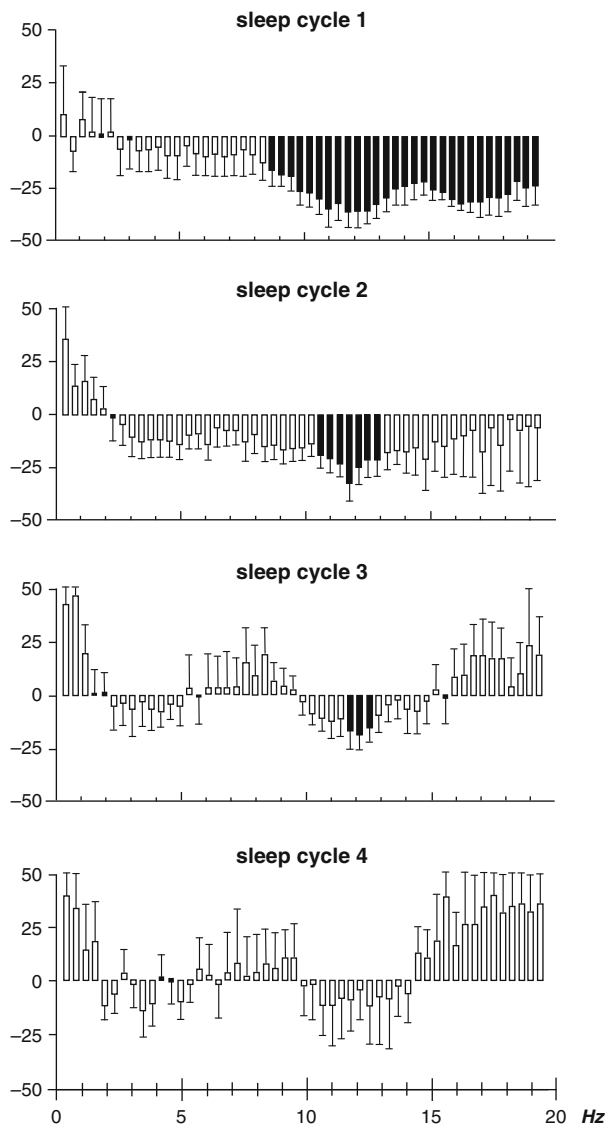


Fig. 5 EEG power spectra (0–19.3 Hz) for sleep cycles 1–4 in young male healthy volunteers (*n* = 6) after pregnenolone. Bars show deviation from placebo level (= 100%) in each 0.39 Hz bin (error bars: ± SEM). Solid bars denote significant differences between placebo and pregnenolone (two-sided Bonferroni *t*-test, nominal *P* < 0.05). From Steiger et al. [91], with permission from Elsevier

flurazepam. No significant interaction with 3 α , 5 α -THDOC and flurazepam was found except an increase in REM latency. These findings suggest an agonistic effect of 3 α , 5 α -THDOC at the GABA_A receptor [94]. The influence of 3 α , 5 α -THDOC on sleep EEG was investigated in rats in comparison to the effects of

allopregnanolone. Intraperitoneal injection of 3α , 5α -THDOC shortened sleep latency, promoted preREM sleep, and prolonged the non-REM sleep episodes dose-dependently. Within non-REM sleep EEG, low frequency activity decreased dose-dependently whereas the activity in the spindle and higher frequency bands increased. The sleep-EEG changes after 3α , 5α -THDOC closely match those of allopregnanolone. This finding indicates a common mechanism of action, probably an agonistic modulation of GABA_A receptor function [95].

6.3 Progesterone

As early as in 1954, a dose-dependent hypnotic effect of intravenous progesterone was reported [96]. After intraperitoneal administration of three dosages of progesterone at dark onset in rats, dose-dependent decreases of sleep latency, wakefulness and REM sleep and increases of REM latency and of preREM sleep were found. In addition, EEG activity decreased in the lower and increased in the higher frequency ranges [97]. To assess the involvement of GABA_A receptor in the sleep response to progesterone, sleep EEG was examined in rats after one dose of the GABA_A antagonist picrotoxin and progesterone, given intraperitoneally alone or in combination. Compared to placebo, picrotoxin significantly delays sleep latency and thereby decreases all sleep stages but has no distinct effects on EEG activity within non-REM sleep. Progesterone shortens sleep latency, increases preREM sleep, lowers frequency EEG activity, and enhances EEG activity in the higher frequencies of non-REM sleep. Except for the changes in the higher EEG frequency range, picrotoxin abolishes all effects of progesterone. These findings suggest that the GABA_A receptor contributes distinctly to the sleep-EEG effects of progesterone [98].

In healthy male volunteers after acute oral progesterone, non-REM sleep, particularly sleep stage 2, increases and SWA decreases [99]. In addition, EEG power in the higher frequency range (>15 Hz) tends to increase. Distinct interindividual variability in the bioavailability of progesterone was found in this study. Consequently, there was also a variability in the time course of the levels of its metabolite allopregnanolone. Therefore, two subgroups were analyzed separately, one during an early peak and one during a late peak of allopregnanolone. Interestingly, the time course of these peaks is associated with the changes in the EEG power spectra. In the subjects with an early allopregnanolone peak, an initial increase in the EEG activity in the spindle and α range during the first few hours of sleep occurs. In contrast, the decrease of SWA was found mainly in those subjects with a later peak of this metabolite (see Fig. 6). The sleep-EEG changes after progesterone in this study are similar to those after agonists at the GABA_A receptor. They appear to be mediated early by conversion of progesterone into allopregnanolone.

After the menopause, progesterone concentrations decline in women. In a crossover design with two treatment intervals of 21 days duration separated by a 2 weeks washout period, an oral dose of 300 mg micronized progesterone was given each for 21 days. In comparison to placebo, progesterone induces a decrease

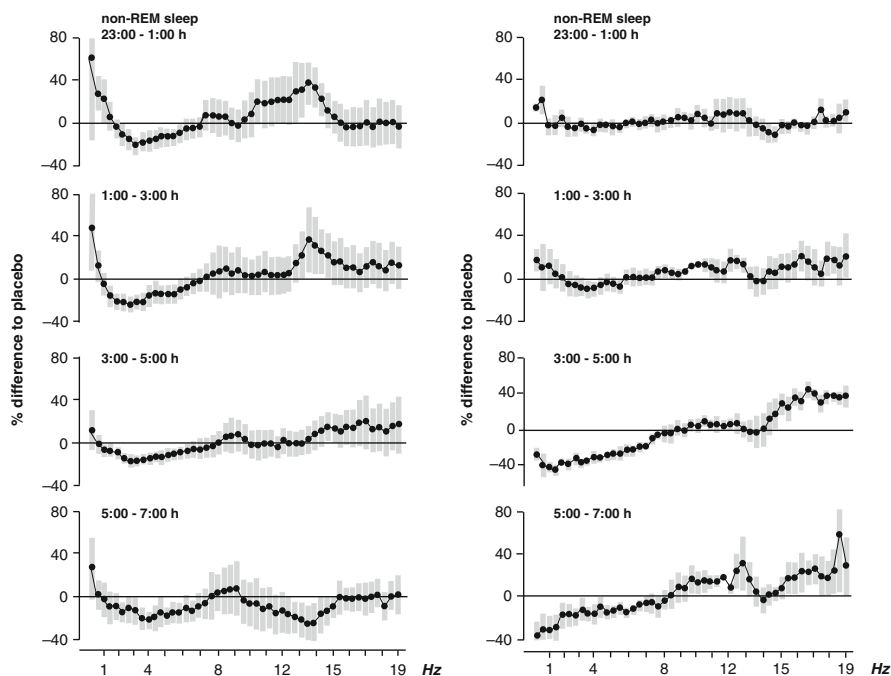


Fig. 6 EEG power spectra of non-REM sleep in young male healthy volunteers after progesterone shown separately for two subgroups of subjects: with an early allopregnanolone peak (*left*; $n = 5$) and a late peak of steroid levels (*right*; $n = 4$). Data represent mean (\pm SEM) deviation from placebo condition (= 100%, depicted by 0 reference line). From Friess et al. [99], used with permission of American Physiological Society

of intermittent time spent awake. During the first third of the night, REM sleep increases. The absolute EEG-power spectra during the total night do not differ between groups. Towards the end of the night, the relative power of EEG δ , σ , and β activity is reduced in comparison to placebo. Beside the decrease of intermittent wakefulness, no typical benzodiazepine-like sleep pattern was found in this study. The effects of continuous progesterone administration over longer time periods seem to differ from the acute effect [100]. The GABA subunit expression might be modified by chronic administration of progesterone [101], whereas daily administration of allopregnanolone during 5 days did not prompt a habituation to its sleep-promoting effects in rats [102].

6.4 Allopregnanolone

The effects of the neuroactive metabolite of progesterone, allopregnanolone on sleep EEG was tested in rats. Two doses of the substance were given intraperitoneally. Compared to placebo, both doses of allopregnanolone shortened sleep latency. The higher dosage significantly increased the time spent in preREM sleep. Furthermore,

EEG activity during non-REM sleep and REM sleep was influenced dose-dependently. During non-REM sleep, EEG activity was decreased in the lower frequencies (≤ 7 Hz) and enhanced in the frequency range of ≥ 13 Hz. In REM sleep, allopregnanolone enhanced high frequency EEG activity (≥ 17 Hz). These effects were most distinct during the first post injections hours. Later on, they declined continuously. Analysis of the plasma and brain concentrations of the substance revealed long-lasting increases, which reached maximal levels during the first post injection hour. The sleep-EEG effects of allopregnanolone are very similar to those after larger doses of progesterone in the rat. The findings suggest that allopregnanolone acts like a benzodiazepine agonist on sleep EEG [103]. In order to investigate the tolerance potential of allopregnanolone, sleep–wake behavior of rats was studied before, during and after treatment with allopregnanolone or placebo for 5 days. Sleep patterns of placebo and allopregnanolone did not differ significantly before and after the treatment. Throughout the total treatment period after allopregnanolone, shorter sleep latencies, prolonged REM latencies, longer non-REM episodes, a larger amount of preREM sleep, reduced low-frequency, and higher spindle activity within non-REM sleep were found than after placebo. In contrast to benzodiazepines the risk of tolerance appears to be low for allopregnanolone [102].

6.5 *Dehydroepiandrosterone*

After a single oral dose of dehydroepiandrosterone (DHEA), REM sleep selectively increases in male healthy volunteers [104]. This observation is compatible with a mixed GABA_A agonistic/antagonistic effect. After intraperitoneal administration of DHEA sulfate (DHEAS), a dose-dependent effect on EEG power was found in rats. A lower dose (50 mg/kg) DHEAS enhances EEG power in the spindle frequency range. In contrast, 100 mg/kg DHEAS exerts the opposite effect. No effect on sleep architecture was found after either dosage of the substance [105].

7 *Neuropeptides*

Various neuropeptides participate in sleep regulation (review: [106]). Some of them appear to act via GABAergic mechanisms.

7.1 *Growth Hormone-Releasing Hormone*

Growth hormone-releasing hormone (GHRH) is the key hormone of the hypothalamo–pituitary–somatotrophic system. Besides stimulating the release of growth hormone, GHRH is also a major sleep-promoting factor. After central and

systemic administration of GHRH, non-REM sleep increases in various species including humans, at least in male subjects and animals. Various studies showed that the amount of non-REM sleep is high when GHRH activity is high and vice versa (reviews: [106, 107]). Calcium levels in GABAergic neurons cultured from rat fetal hypothalamus increase when they are perfused with GHRH. Many hypothalamic GHRH responsive neurons appear to be GABAergic [108]. The hypothesis was tested whether GHRH promotes non-REM sleep via activation of GABAergic neurones in the preoptic area. Groups of rats received intracerebroventricular injections with GHRH or placebo at the onset of the dark period and were permitted to sleep spontaneously for 90 min. Separate groups of rats were sleep-deprived for 90 min, beginning at the time of GHRH or placebo injection. Other groups received the somatostatin analog octreotide or a competitive GHRH antagonist, or placebo at light onset. Their spontaneous sleep–wake behavior was recorded for 90 min. Rats were killed after the 90 min monitoring period. Brain tissue was processed for immunohistochemistry for c-Fos protein and glutamic acid decarboxylase (GAD). Single c-Fos and dual Fos-GAD cell counts were performed in the median preoptic nucleus (MnPN), and in the core and the extended parts of the ventrolateral preoptic nucleus (cVLPO and exVLPO). Non-REM sleep increased after GHRH. Double-labeled Fos + GAD cell counts were elevated significantly after GHRH in the MnPN and VLPO in both undisturbed and sleep-deprived groups. Octreotide and the GHRH antagonist significantly decreased the amount of non-REM sleep compared to control animals. After octreotide, single c-Fos-labeled cell counts increased in the MnPN but not in the VLPO. Double-labeled cell counts were significantly reduced after octreotide and the GHRH antagonist in all areas examined. These findings suggest that GABAergic neurons in the MnPN and VLPO are potential targets of sleep-regulatory actions of GHRH [109].

7.2 *Neuropeptide Y*

After intracerebroventricular administration of neuropeptide Y (NPY) in the rat, changes of EEG spectral activity are found, which resemble those after benzodiazepines [110]. The prolongation of sleep latency after the sleep-impairing peptide corticotropin-releasing hormone is antagonized in a dose-dependent fashion by NPY in rats [111]. In healthy male volunteers, after repetitive iv injections around sleep onset, sleep latency and the duration of the first REM period decrease whereas the time spent in sleep stage 2 and the sleep period time increase. All these changes are similar to sleep-EEG effects of benzodiazepines [112]. In patients with depression of both sexes with a wide age range and in matched controls, sleep latency decreases whereas other sleep-EEG variables remain unchanged [113]. It is thought that NPY participates in sleep regulation, particularly as a signal for sleep onset as an antagonist CRH acting via a GABA_A receptor.

7.3 *Galanin*

The neuropeptide galanin is widely distributed in the mammalian brain. A cluster of GABAergic and galaninergic neurons was found in the ventrolateral preoptic area, which is thought to stimulate non-REM sleep [114]. This hypothesis is supported by the observation that after pulsatile iv injections of galanin to young male healthy volunteers, SWS and the duration of REM periods increase [115].

8 Conclusions and Perspectives

As reviewed in this chapter, various synthetic and endogenous ligands of the GABA_A receptor are capable to modulate sleep–wake behavior in a specific fashion. Their effects range from sleep promotion by agonists to sleep impairment after inverse agonists. The most frequently used hypnotics worldwide today are benzodiazepines and the so-called nonbenzodiazepine hypnotics. Generally, these substances help to initiate and to maintain sleep. However, their effects on the sleep EEG, particularly the microstructure of sleep, are opposite to the effects of sleep deprivation. Sleep deprivation is the most powerful method to promote sleep [116]. The amounts of SWS and REM sleep increase. Sleep-EEG power increases in the lower frequency range and decreases in the higher frequency range. One should expect that ideal hypnotics share the effects of sleep deprivation. However, after benzodiazepines and nonbenzodiazepine hypnotics as well, opposite changes of sleep EEG occur, in detail decreases of SWS, REM sleep, and sleep EEG in the lower frequency range together with increases of power in the higher frequency range. Sleep-EEG changes resembling those after sleep deprivation were found after gaboxadol and tiagabine. The development of gaboxadol as hypnotic was stopped. Tiagabine is approved as anticonvulsant only so far. Furthermore, the neurosteroids pregnenolone and the neuropeptides GHRH and galanin influence sleep in a similar fashion like sleep deprivation. GHRH is even thought to be involved in the sleep-promoting action of sleep deprivation [107]. Another interesting observation is the improvement of sleep after progesterone replacement in menopausal women [100]. These observations are leads for the development of novel hypnotics, which are better related to human physiology than the drugs available so far. It should be kept in mind also that some endogenous substances (progesterone after acute administration, allopregnanolone, 3 α , 5 α -THDOC, and NPY) share the sleep-EEG effects of benzodiazepines. Sleep regulation is a complex interplay of various sleep-promoting and sleep-impairing factors including beside of peptides and steroids classical monoaminergic neurotransmitters, adenosine, orexin, and cytokines. The exact role of neurosteroids acting at the GABA_A receptor in sleep regulation is not yet understood.

In the 1980s, research in pharmaceutical companies addressed the various actions of ligands of the GABA_A receptor (see for example [83]). However, except

for flumazenil, no drugs were developed as a consequence of this research. One could imagine that certain partial inverse agonists may be helpful in hypersomnic disorders like narcolepsy and sleep apnea. The finding that REM sleep is increased after DHEA may be of clinical relevance since REM sleep is thought to play a role in memory consolidation. An interesting hypothesis is the view that DHEA substitution may be a treatment for cognitive impairment. Finally, the use of benzodiazepine antagonists was shown to augment the antidepressant effect of sleep deprivation [82]. This finding may help to develop a novel strategy in the treatment of affective disorders. In all, research in the field of sleep–wake regulation by substances acting at the GABA_A receptor will remain a promising field.

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Subcortical Neuromodulation of Feedforward and Feedback Inhibitory Microcircuits by the Reticular Activating System

J. Josh Lawrence

Abstract GABAergic inhibition originates from distinct subclasses of interneuron subtypes. These interneuron subtypes are highly specialized, exhibiting distinct pre- and postsynaptic properties. The convergence of these specializations enables each interneuron subtype to make a unique but complementary contribution to the function of cortical microcircuits. The existence of morphologically and physiologically distinct interneuron classes raises the possibility of their being controlled independently of one another. Indeed, based on mounting evidence from Somogyi and colleagues for distinct interneuron classes, Nicoll presented a hypothesis that different neuromodulatory systems might exert precise control over principal cell excitability by engaging different interneuron subtypes. Although our knowledge of the manner in which defined interneuron subtypes undergo neuromodulation remains incomplete, there is accumulating evidence that neuromodulatory receptors are differentially expressed on specific GABAergic interneuron subtypes. Therefore, the efficacy of specific GABAergic feedforward and feedback inhibitory circuits will be dynamically regulated by fluctuations in the concentrations of monoamines occurring across the sleep/wake cycle. During periods of wakefulness, alertness, and attention, I propose that the activation of the reticular activating system will engage specific interneuron subtypes in a cell-type specific manner, collectively optimizing sensory processing and information storage in neural microcircuits.

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1 Introduction

In most brain areas across the central nervous system, GABAergic inhibition is an essential component of neuronal microcircuits. GABA_A receptors are a major target for treatment of sleep disorders such as insomnia, but given the ubiquity of GABA_A receptors and the complexity of neural circuits involved in regulating behavioral states, it is easy to become lost in the vastness of potential cellular and synaptic targets where GABA_A receptor modulators might act to influence waking and sleeping states. It is especially intimidating to think of GABAergic inhibition in the historic view as generic synapses that release GABA onto postsynaptic GABA_A receptors. However, within the last 15 years of scientific investigation, the concept of a generic GABA synapse has been largely abandoned, ushering in an era where GABAergic inhibitory interneurons are viewed as some of the most specialized of neuronal types in the brain. Specific subtypes of GABAergic interneuron populations differ morphologically, electrophysiologically, and synaptically. Moreover, the composition of GABA_A receptors differs across GABAergic cell types. The convergence of these specializations enables each interneuron subtype to make a unique but complementary contribution to the function of cortical microcircuits. At the same time, this heterogeneity also offers an opportunity for therapeutic intervention with drugs that act at specific types of GABA synapses.

Synchronous activation of populations of neurons can induce rhythms between reciprocally connected excitatory and inhibitory neurons. The magnitude and frequency of neuronal rhythms, or oscillations, differs widely across wake and sleep states, reflecting temporal dynamics in the number and type of neurons engaged at a particular point in time. The activity of neuromodulatory neurons of the reticular activating system (RAS) is thought to play a central role in these neuronal rhythms. During periods of wakefulness, alertness, and attention, the activation of RAS neurons releases monoamines that may serve to optimize sensory processing and information storage in neural microcircuits, manifested behaviorally as arousal and attention. In addition to principal cells, GABAergic interneurons are also regulated by these neuromodulatory inputs, as well as local neuromodulators such as by endocannabinoids and neuropeptides. Our knowledge of how defined interneuron subtypes undergo neuromodulation remains incomplete, but evidence is growing that specific GABAergic interneuron subtypes respond differentially to neuromodulation. Therefore, the efficacy of specific GABAergic feedforward and feedback inhibitory circuits will be dynamically regulated by fluctuations in the concentrations of monoamines occurring across the sleep/wake cycle. Moreover, GABAergic projection neurons can mediate long-distance signaling between brain nuclei, enabling the synchronization of neuronal oscillations across brain regions.

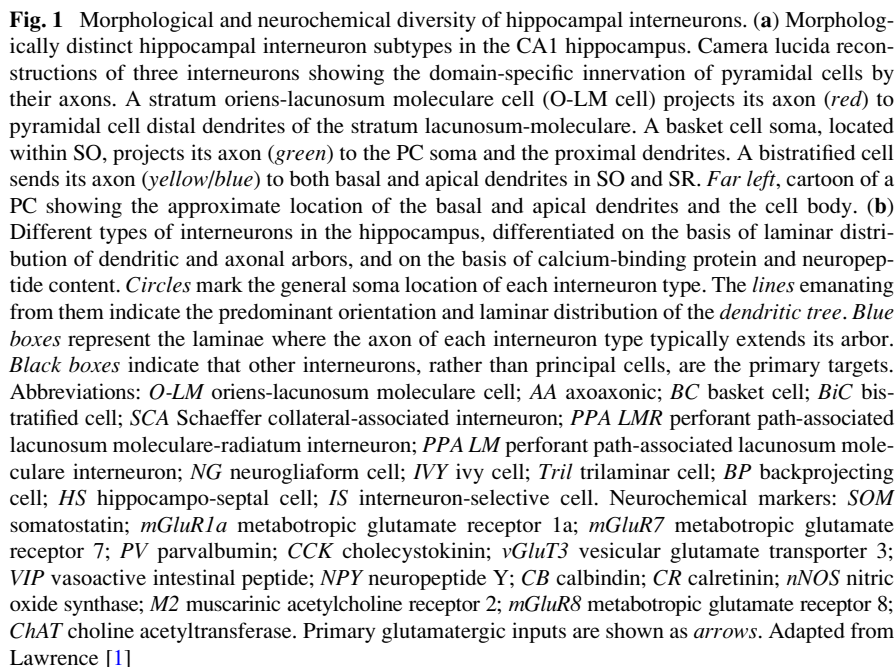
In this chapter, I demonstrate that the nature of inhibition takes many forms, by examining specific GABAergic interneuron cell types in detail. These interneuron subtypes possess specific pre- and postsynaptic specializations to provide feedforward and feedback inhibition along different spatial and temporal dimensions. I will

limit my description of the major cell types to area CA1 of the hippocampus, though analogous cell types are present in neocortex and other parts of the brain. Finally, to gain an appreciation of how specific GABAergic circuits may be altered during wakefulness and sleep, I will examine our current understanding of the neuromodulatory specializations of these GABAergic interneuron subtypes.

2 GABAergic Inhibition Originates from Distinct Subclasses of Interneuron Subtypes that Exhibit Both Pre- and Postsynaptic Specializations

A large diversity of GABAergic interneurons exist (Fig. 1). Compiled from *in vivo* and *in vitro* experiments, interneuron subtypes fall into different categories based on morphological, neurochemical, electrophysiological, synaptic, and/or network criteria. Although it is difficult, if not impossible, to acquire all of these parameters simultaneously to identify an interneuron as part of a specific subclass, it is becoming increasingly necessary to employ multiple criteria (i.e., both neurochemical and anatomical) to unambiguously assign a cell to a particular subclass. Many cell types have been classified based on *in vivo* criteria, such as the firing of the cell during network oscillations, combined with post-hoc anatomical and immunocytochemical characterization. *In vitro* criteria, such as passive properties, active conductances, synaptic properties, as well as anatomical and firing of cells to artificially induced oscillations, also serve to classify cells. Although both *in vivo* and *in vitro* preparations permit a post-hoc immunocytochemical analysis of neurochemical content, *in vitro* preparations also permit the technique of single cell PCR, in which one can obtain a fairly complete neurochemical profile of the neuronal identity that is not bounded by the number of fluorophores or slices that contain the neuron of interest. Finally, mouse transgenic technology and viruses enable fluorophores to be expressed in neurochemically restricted subsets of GABAergic interneurons. This new technology permits a more systematic examination of specific interneuron subtypes.

From these *in vivo* and *in vitro* experiments, GABAergic interneurons are classified into broad classes based on neurochemical, anatomical, and electrophysiological criteria [2], as well as the manner in which they function within networks. Neurochemical criteria are rapidly expanding, but interneuron subpopulations can be distinguished based on calcium-binding proteins (parvalbumin, calbindin, calretinin), neuropeptide content (somatostatin (SOM), cholecystokinin (CCK), neuropeptide Y (NPY), vasoactive intestinal peptide (VIP)), and cell-specific enzymes or receptors (nitric oxide synthase, metabotropic glutamate receptor 1, M2 muscarinic receptor). Anatomical criteria are currently classified based on orientation of the dendrites, laminar localization of the cell body, presence or absence of dendritic spines, and spatial distribution of the axonal arborization. Two major anatomical cell types exist, perisomatically targeting and dendritically targeting cells. Electrophysiological



criteria can be used to classify interneurons into categories such as fast-spiking, adapting, and stuttering. These firing phenotypes are shaped by both passive and active properties of each cell type.

Finally, interneurons can be classified as feedforward, feedback, or both, based on the configuration of their synapses within networks. Interneurons that participate in feedforward inhibition receive a common extrinsic glutamatergic afferent and are in a position to provide “feedforward” inhibition to target glutamatergic cells (Fig. 2a, ff). Interneurons that participate in feedback inhibition receive intrinsic glutamatergic afferents from the target glutamatergic cell itself (Fig. 2a, fb). Many interneuron subtypes can participate in both feedforward and feedback inhibition, though there may be some that are “pure” feedforward or feedback interneurons. A discussion of specific classes of interneurons follows.

2.1 *Parvalbumin-Positive Basket Cells*

Parvalbumin-positive (PV+) basket cells (BCs) innervate the perisomatic regions of CA1 pyramidal cells, providing a powerful GABAergic input ideally suited to control the output and synchronization of principal cell populations [4]. PV+ neurons receive a higher density of glutamatergic synapses than pyramidal cells or PV-negative interneuron subtypes [5]. As a consequence, excitatory postsynaptic currents (EPSC) are larger in PV BCs than in other cell types, enabling them to fire more readily in response to Schaeffer collateral input [3] and during sustained oscillatory activity [6] (Fig. 2). PV BCs have an obvious fast-spiking electrophysiological phenotype, referring to their ability to fire at high frequencies. This feature is due to the high expression of Kv3 channels [7] that allow rapid repolarization, narrow action potential half-width [8], and spike frequency preference in the gamma frequency range [9].

The heavy integration of PV BCs into glutamatergic networks [3, 5], low input resistance [3], rapid repolarization of the action potential [7, 8], and brief inhibitory postsynaptic current (IPSC) [10, 11] in the postsynaptic cells allow PV BCs to follow high frequency neuronal oscillations, and, most recently, has been demonstrated to shape gamma oscillations [12]. The suprathreshold action potential in the PV BCs propagates to the presynaptic terminals, releasing GABA onto the perisomatic regions of the CA1 pyramidal cells. As a consequence of stimulation of Schaeffer collaterals from CA3 pyramidal cells, the overall result of glutamatergic input is a monosynaptic EPSC in CA1 pyramidal cells followed by a disynaptic GABAergic IPSC (Fig. 2e, f). Therefore, the glutamatergic excitation of pyramidal cells is restricted to a brief time window between the arrival of glutamatergic input and GABAergic input [13]. Interestingly, the postsynaptic densities apposed to BC synapses are enriched in $\alpha 1$ GABA_A receptors [14], which are associated with rapid deactivation kinetics and sensitivity to the sleep medication zolpidem.

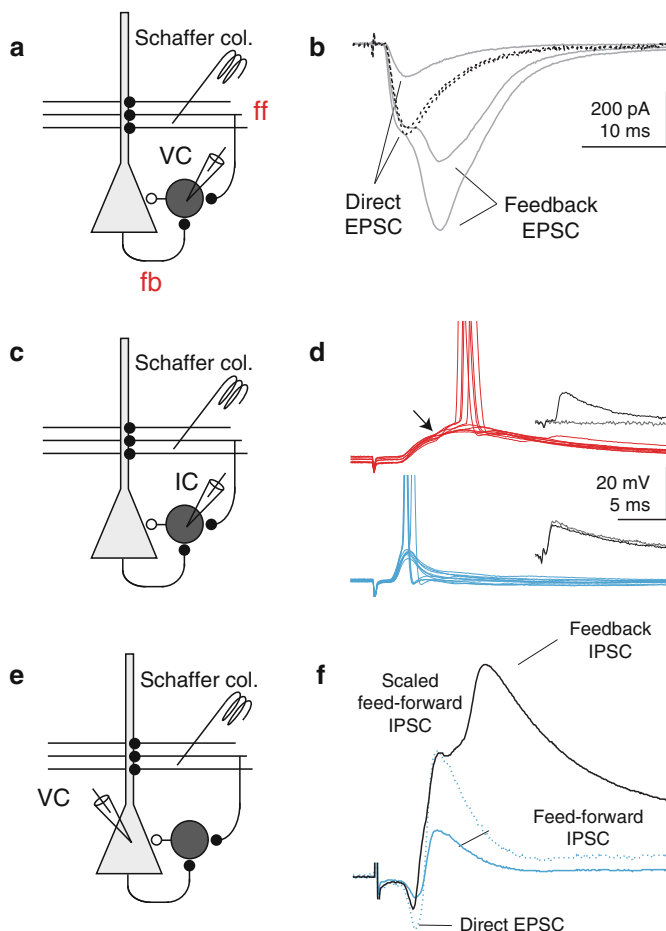


Fig. 2 Feedforward and feedback inhibition mediated by PV and CCK BCs. **(a)** Recording configuration featuring Schaffer collateral (SC) stimulation onto a recorded interneuron in voltage clamp (VC) mode. **(b)** Voltage-clamp recording from an interneuron in response to Schaffer collateral stimulation at three different intensities (2.5 μ M gabazine). Note the appearance of a late, feedback EPSC at stronger stimulation intensities. *Dotted trace*, the EPSC recorded at low stimulus intensity, is scaled to the peak of the early component elicited at strong stimulation intensities. **(c)** Recording configuration featuring SC stimulation onto a recorded interneuron in current clamp (IC) mode. **(d)** Ten superimposed current traces from CCK (red) and PV (blue) basket cells at threshold for spiking in response to SC stimulation. Action potentials have been truncated. Note the discontinuity (arrow) in the rise of the EPSP in the CCK basket cell, due to the onset of the feedback EPSP. **(e)** Recording configuration featuring SC stimulation onto a CA1 pyramidal cell in VC mode. **(f)** VC recording from a pyramidal cell in response to SC stimulation at two different intensities (blue trace, low intensity; black trace, high intensity). Note the appearance of a late, feedback IPSC at the stronger stimulation intensity. *Dotted trace*, the feedforward IPSC elicited at low stimulation intensity scaled to the peak of the feedforward IPSC elicited at high intensity. From Glickfeld and Scanziani [3]

2.2 Axoaxonic Cells

Axoaxonic cells (AAs), also called chandelier cells, are PV+ cells that are distinct from PV BCs, in that they innervate the axon initial segment of CA1 pyramidal cells [15]. In contrast to dendrite-targeting cells, in which GABAergic terminals regulate synaptic inputs, AAs are thought to control the output (spike generating center). As with PV BCs, synapses established by AA are enriched in $\alpha 1$ GABAA receptors [14]. Although still debated, it is likely that the chloride concentration in the axon is regulated differently than in the dendrites, causing the IPSPs from AAs to be depolarizing in nature [16, 17] but see [18].

2.3 CCK BCs and Schaeffer Collateral-Associated (SCA) Cells

A second type of perisomatically targeting cell is the CCK BC. CCK BCs are a nonoverlapping population with PV BCs, distinguished by the presence of presynaptic CB1 receptors that confer sensitivity to endocannabinoids [19] (Fig. 3). Moreover, the temporal dynamics of GABA release at these synapses feature a prominent asynchronous component [21, 22]. CCK BC synapses are enriched in $\alpha 2$ GABA_A receptors. Because these cells receive a lower density of glutamatergic afferents than PV BCs, it is rare that glutamatergic input from a single pathway elicits action potential firing. This makes it harder for CCK BCs, relative to PV BCs, to be engaged by feedforward excitation [3]. However, these cells readily achieve threshold through activation of multiple excitatory pathways, such as during strong synaptic stimulation when feedback excitation follows Schaeffer collateral stimulation (Fig. 2c, red). The delay in the synaptically activated action

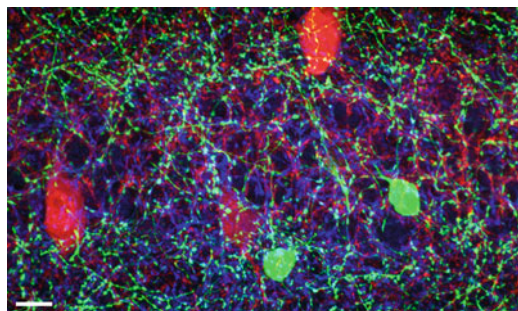


Fig. 3 Distinct CB1+ and PV+ terminals in the CA1 pyramidal cell layer. Triple labeling of GAD65-GFP from a transgenic mouse [20], CB1 labeling (blue), and PV (red) CB1 and PV. The following antibodies were used CB1 receptor (rabbit primary: 1:10,000, Cayman Chemical; secondary: goat antirabbit Alexa 405, Invitrogen) and PV (mouse primary: 1:1000, Sigma; secondary: goat antimouse Alexa 633). Confocal stacks were acquired and flat projected with an Olympus Fluoview 1000 confocal microscope. Scale bar: 10 μ m

potential in the CCK BC translates to a feedback IPSC in the CA1 pyramidal cell (Fig. 2e). This feedback IPSC is regulated by endocannabinoids released by CA1 pyramidal cells in an activity-dependent manner. Therefore, the efficacy of CCK interneuron inhibition is highly regulated by the degree of ongoing activity in principal cells (Fig. 2f). CCK BCs are likely tuned to encode the onset of excitatory synaptic stimuli, being transiently recruited during a train of excitatory inputs due primarily to their short-term depression at their Schaeffer collateral synapses. Schaeffer collateral-associated (SCA) cells, a second class of CB1 receptor-expressing CCK cells that target the dendritic regions of CA1 pyramidal cells, may play a similar role in the CA1 circuit. CCK BCs and other CCK cells may be partially or entirely muted by tonic levels of endocannabinoids that persistently activate CB1 receptors [23, 24].

2.4 *Somatostatin-Positive Interneurons*

Somatostatin-positive (SOM+) cells, such as the oriens-lacunosum moleculare (O-LM) cells in stratum oriens of CA1, project to the distal dendrites of CA1 pyramidal cells [92, 93], where they are thought to gate distal entorhinal input to CA1. O-LM cells have dendrites that are contained predominantly within stratum oriens, therefore receiving minimal input from Schaeffer collaterals and the most from CA1 collaterals. For this reason, these cells have been thought to mediate solely feedback inhibition. However, other somatostatin cells that lie in the pyramidal cell layer and stratum radiatum (termed P-LM and R-LM cells, respectively) are likely to receive a mixture of Schaeffer collateral and CA1 synaptic input, yet they also project distally [25]. Thus, it is likely that many SOM+ cells function to gate distal entorhinal input. The time course of IPSCs evoked from O-LM cells is likely shaped by both their distal electrotonic location [26] and probably to the slower time course of the inhibitory conductance [27]. Upon repetitive activation of the alveus, EPSCs onto these cells undergo robust short-term facilitation, which is in contrast to the transient nature of synaptic activation onto perisomatically targeting neurons [28]. When repetitively activated by excitatory input, EPSCs become suprathreshold later in the train. Thus, onset and rate of sensory stimuli appear to be encoded by different interneuron subtypes that control distinct surface domains of CA1 pyramidal cells.

2.5 *Neurogliaform Cells*

Another type of dendritically projecting cell that has recently been well characterized is the neurogliaform cell [29]. Like the dendritically targeting somatostatin cells of hippocampus and cortex, these cells elicit a slow IPSC in pyramidal cells that probably have a component of electrotonic filtering. However, in addition to

GABA_A receptors, GABA_B receptors are also activated, demonstrating that GABA_B receptors can be synaptically activated by single action potentials. The IPSCs elicited from neurogliaform cells undergo short-term depression. A low affinity GABA_A receptor competitive antagonist TPMPA revealed that, compared to conventional PV BC synapses, the peak GABA transient in the synaptic cleft is lower and has a prolonged time course [30]. Of particular relevance to sleep research, zolpidem, which enhances the affinity of $\alpha 1$ GABA_A, had a potent effect on this synapse, greatly potentiating the amplitude of the IPSC generated by neurogliaform cell activation [30] but, in contrast to PV BCs, had no effect on the time course of the IPSC. A similar cell type has been reported in the hippocampus that is associated with slow, readily depressing GABA_A-mediated IPSCs [31, 32].

3 Cell Type-Specific Control of Feedforward and Feedback GABAergic Circuits by the Reticular Activating System

One important consideration is that many neuromodulator systems in the RAS, such as serotonin, histamine, acetylcholine, and norepinephrine, are differentially active during wake and sleep states. The afferents project extensively to hippocampus and neocortex where they alter cellular and synaptic microcircuits, manifested behaviorally as arousal, wakefulness, and attention. There is growing evidence that these neuromodulatory systems impact specific GABAergic networks. Therefore, waking and sleeping behavioral states will likely alter GABAergic inhibition through neuromodulation of specific GABAergic networks.

3.1 Cholinergic Modulation of GABAergic Circuits by the Basal Forebrain

In many GABAergic interneurons, pharmacological activation of muscarinic acetylcholine receptors (mAChRs) results in a membrane depolarization [33–37] that is somewhat reminiscent to that seen in pyramidal cells [38, 39]. However, there is wide variation across hippocampal interneurons to activation of mAChRs [33, 35, 36, 40]. In contrast to the slow sustained modulation produced by mAChR activation in both pyramidal cells and interneurons, puff application of nicotinic acetylcholine receptor (nAChR) agonists produce a more transient response in hippocampal interneurons [41, 42]. Similar to neocortical interneurons [43–46], there is also growing evidence for cell type specificity in postsynaptic expression of nAChRs across hippocampal interneuron subtypes (Fig. 4). The emerging picture is that hippocampal interneuron subtypes exhibit different responses to acetylcholine depending on their neurochemical identity and cholinergic receptor subtype composition [1, 36, 37, 47].

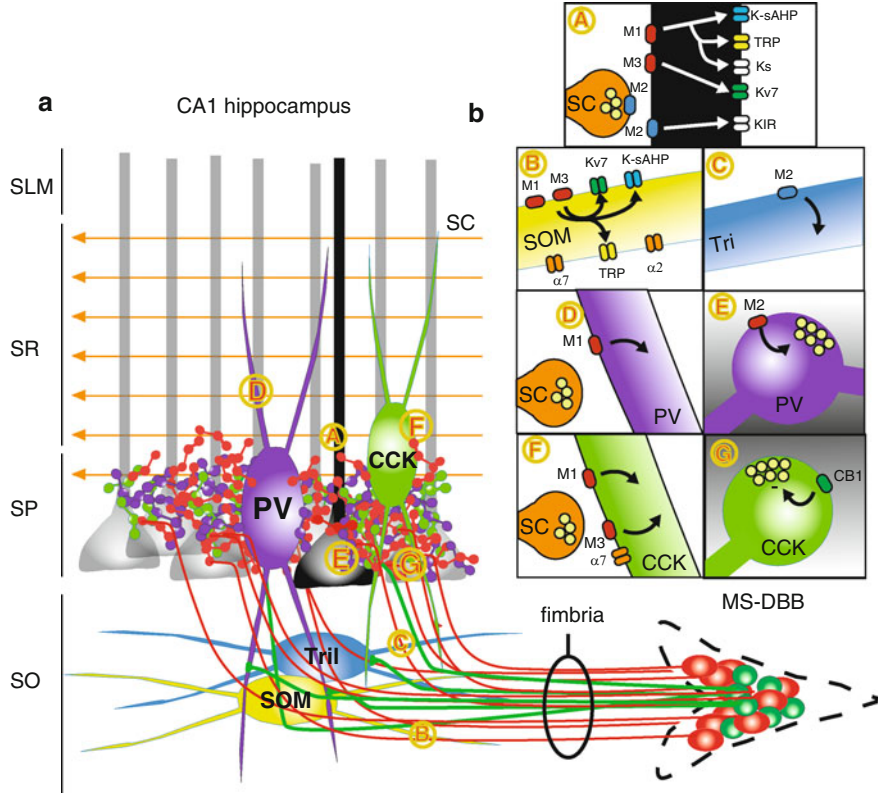


Fig. 4 The medial septal-diagonal band of Broca (MS-DBB) projection to defined cellular and synaptic targets of the CA1 hippocampus. **(a)** The MS-DBB is composed of cholinergic (red) and GABAergic (green) neurons that project via the fimbria to hippocampal regions. Cholinergic projection fibers (red) pass through stratum oriens (SO), where the somatostatin (SOM)-positive oriens-lacunosum moleculare (O-LM) neurons (yellow) and trilaminar (blue) interneurons are located, and arborize in a dense network within stratum pyramidale (SP) with CA1 pyramidal cells (black), CCK BCs, and PV BCs (cholinergic terminals in stratum oriens and stratum radiatum (SR) omitted for clarity). MS-DBB GABAergic neurons (**a**, green cells) are thought to innervate exclusively hippocampal interneurons. Areas of interest, denoted by circled numbers in **a**, are expanded in **b**. Known cellular and synaptic targets, denoted by circled numbers, are shown. These are **(a)** the dendrites of pyramidal cells, acting at M1, M2, and M3 mAChRs and presynaptic terminals of Schaffer collaterals (orange) acting at M2 mAChRs **(b)** somatodendritic regions of O-LM cells acting at M1 mAChRs, M3 mAChRs, $\alpha 7$ nAChRs, and non- $\alpha 7$ nAChRs, **(c)** somatodendritic regions of trilaminar interneurons acting at M2 mAChRs, **(d)** somatodendritic regions of PV BCs acting at M1 mAChRs, **(e)** presynaptic terminals of PV BCs acting on M2 mAChRs, **(f)** Somatodendritic regions of CCK BCs acting on M1 and M3 mAChRs and $\alpha 7$ nAChRs, and **(g)** presynaptic terminals of CCK BCs acting indirectly through presynaptic CB1 mAChRs

3.1.1 O-LM Cells

O-LM cells are a subclass of hippocampal interneuron [48] that generate a large, highly stereotypical response to mAChR activation [1, 36, 49], very much

paralleling the unusual sensitivity of another (SOM+) interneuron subtype, the neocortical Martinotti cells, to cholinergic activation [50, 51]. In the presence of muscarine or ACh, O-LM cells respond to suprathreshold current steps with an acceleration in firing frequency followed by a prominent suprathreshold afterdepolarization (ADP) [36], reminiscent of the cholinergic plateau potentials seen in CA1 pyramidal cells [39]. Switching to voltage clamp after the depolarizing stimulus revealed that a nonselective cationic current (I_{CAT}) underlies the ADP, which is most likely mediated by TRPC channels [38]. Moreover, mAChR activation of O-LM cells results in the inhibition of both M- (I_{M}) and slow afterhyperpolarization K+ currents (I_{AHP}) [36, 52]. The mAChR response in O-LM cells was reduced in M3 KO mice but fully eliminated in M3 KO mice preincubated with an M1 antagonist, suggesting that M1 and M3 mAChRs contribute to mediating mAChR responses in these cells [36]. However, using an M1 antibody and in situ hybridization, Watanabe and colleagues recently found little evidence for strong M1 mAChR expression in putative SOM+ and mGluR1+ cells in stratum oriens [53]. Therefore, the mAChR composition is still not definitively established in O-LM cells. Importantly, in SO interneurons that were distinct in morphology from O-LM cells, the mAChR-induced ADP did not occur, demonstrating that mAChR responsiveness depends on interneuron subtype [36]. mAChR modulation also enhances the intrinsic oscillatory properties of O-LM cells at theta band (5–12 Hz) frequencies [49]. Inhibiting I_{M} in O-LM multicompartmental models reproduced this observation [1, 52]. In response to application of nAChR agonists, O-LM cells generate nicotinic currents mediated by both fast $\alpha 7$ and slow non- $\alpha 7$ nAChRs [42, 54]. Also, $\alpha 2$ -containing nAChRs on stratum oriens cells exhibit sustained nAChR activation, which contributes to robust nAChR-mediated inhibition of principal cells [55].

3.1.2 PV BCs

In previous work, PV+ BCs did not exhibit strong excitation by nAChRs or mAChR activation in the neocortex [43, 46, 50] or hippocampus [35, 54]. However, with the use of PV-GFP mice, we recently revisited the mAChR sensitivity of hippocampal PV+ BCs [37]. In response to mAChR activation, PV BCs depolarize, increase in firing frequency, and exhibit a loss of an afterhyperpolarization, all of which are lost in M1 KO mice [37]. Moreover, single cell PCR revealed M1 mAChRs to be a predominant mAChR subtype in these cells [37]. Interestingly, upon bulk fiber stimulation with a large stimulating electrode in stratum oriens, a detectable response was observed in CCK BCs but not PV BCs [37]. However, upon bath application of tacrine, mAChR-sensitive responses were generated upon bulk fiber stimulation, suggesting that M1 mAChRs present on PV BCs are positioned mainly for volume transmission. However, given this modest mAChR response, the level of M1 mAChR is probably lower in PV BCs than in CA1 pyramidal cells or CCK BCs [53]. Nevertheless, substantial high levels of ACh

would be expected to engage the PV BC network, which may contribute to the generation of gamma oscillations [94, 95].

3.1.3 CCK BCs and CCK SCA Cells

Using a GAD65 GFP transgenic mouse line in which GFP is expressed in interneurons lacking PV [20, 22, 37], CCK BCs have recently been shown to respond to mAChR activation differently than PV BCs [37]. Similar to that seen in O-LM cells, a prominent mAChR-induced ADP is observed in CCK BCs (Fig. 5). However, this ADP is slower in time course than the O-LM ADP and is often interrupted by a brief, mAChR-insensitive fast afterhyperpolarization that occurs after the offset of a suprathreshold current step. One interesting feature of CCK BCs is that M1 and M3 mAChRs appear to control different mechanisms of excitability. M3 mAChRs control mAChR-induced changes in firing; however, both M1 and M3 mAChRs control the emergence of the mAChR-induced ADP [37]. Therefore, the expression of M3 mAChRs in CCK BCs distinguishes it from PV BCs. CCK-positive SCA interneurons are similar in their response to mAChR-induced neuromodulation, also exhibiting an mAChR-induced ADP [56].

There is some evidence that CCK interneurons are enriched in nAChRs. $\alpha 7$ nAChR and CCK mRNA and protein levels are highly colocalized [57, 58]. Interneurons that straddle the radiatum/lacunosum molecular border exhibit fast $\alpha 7$ nAChR-mediated responses to pharmacological [41, 42, 59] and synaptic [60] activation. GAD65 GFP interneurons that straddle the radiatum/lacunosum

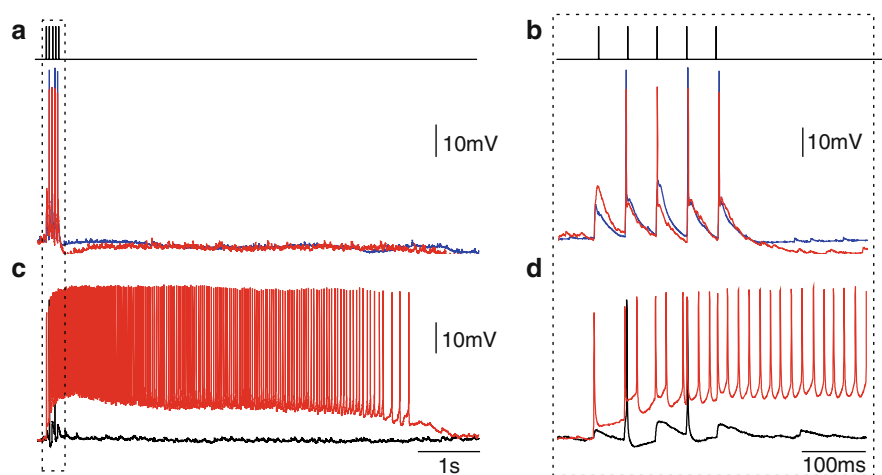


Fig. 5 Glutamatergic excitation of CCK BCs is enhanced by mAChR activation. Representative traces of a train of five EPSPs evoked onto (a, b, blue) a PV BC or (c, d, black) CCK BC under control conditions or after bath application of 10 μ M muscarine (red). Black vertical lines in a and c indicate the onset of synaptic stimulation. Expanded regions during the EPSP train are delineated by dashed boxes in b and d

molecular border are enriched in CCK content [37], consistent with $\alpha 7$ nAChR and CCK colocalization. Therefore, the preponderance of the evidence suggests that the three neurochemically distinct classes of interneurons, CCK, SOM, and PV, also differ on the basis of their nAChR-mediated response profile.

3.1.4 M2-Expressing Trilaminar Cells

There is a population of stratum oriens cells in which M2 mAChR is expressed somatodendritically [61]. Recent immunocytochemical studies show that mGluR1a+ and M2+ SO interneurons are distinct cell types [62] that likely correspond to O-LM and trilaminar cells. Coupled with the known pharmacology of mAChR subtypes on O-LM cells [36], these observations suggest that SO interneuron subtypes possess a different complement of postsynaptic mAChRs. M2+ SO cells are electrically interconnected through dendritic gap junctions [61]. Also in stratum oriens, there is a subpopulation of GABAergic interneuron that exhibits reduced excitability to mAChR activation and no mAChR-induced ADP [36]. Although the functional consequence of M2 mAChR activation in trilaminar cells is still not known, M2 mAChR activation could result in a reduction in input resistance and cellular excitability [36] through inward rectifier potassium channels [35, 63].

3.1.5 Presynaptic Mechanisms of Cholinergic Modulation of Hippocampal GABAergic Circuits

In addition to direct postsynaptic effects of ACh on hippocampal interneurons, GABAergic interneurons also undergo presynaptic inhibition [64, 65]. Bath application of mAChR agonists increases the frequency and amplitude of spontaneous IPSCs, consistent with postsynaptic excitation of hippocampal interneurons. However, the amplitude of monosynaptically evoked IPSCs and the frequency of miniature IPSCs are concomitantly depressed [64, 65]. Which hippocampal interneuron subtypes undergo presynaptic cholinergic modulation? Work by Hajos and colleagues provided evidence that the presynaptic axon terminals of PV+ basket cells express M2 receptors [61]. Consistent with this observation, carbachol-mediated inhibition of IPSCs evoked from the pyramidal cell layer is largely eliminated in M2 knockout mice [66]. In dual-paired recordings from PV BCs and CA3 pyramidal cells, mAChR activation reduces the amplitude of IPSCs from PV + BC terminals [67, 97] which is reversed by the M2 antagonist AF/DX 116 [97]. However, mAChR modulation still remains in M2 knockout mice [67], raising the possibility that additional mAChRs may be present on PV BC terminals. Whether presynaptic terminals of somatostatin- or calretinin-positive interneuron subtypes also undergo presynaptic modulation has not yet been fully elucidated. Interestingly, at CCK+ BC terminals, mAChR activation also inhibits GABA release [23, 97]. However, mAChR modulation

is indirect, in which activation of M1/M3 mAChR on pyramidal cells induces postsynaptic release of endocannabinoids, which diffuse to presynaptic CB1 receptors on CCK BC terminals [23, 68, 69, 97]. As mentioned previously, PV BC terminals do not possess presynaptic CB1 receptors (Fig. 3). Therefore, mAChR-induced modulation GABA transmission at PV BC and CCK BC synapses employ different mechanisms, whereby mAChR-induced modulation of CCK BCs is tied to the activity level of CA1 pyramidal cells through endocannabinoid signaling but mAChR modulation of PV BCs is not [19]. Therefore, cholinergic neuromodulation can alter the efficacy of GABAergic transmission through both pre- and postsynaptic mechanisms.

3.2 Serotonergic Modulation of GABAergic Circuits by the Dorsal Raphe

The raphe nuclei are thought to be part of the RAS, in which activity of raphe neurons is correlated with waking and arousal. A subset of raphe neurons are phase-locked to the hippocampal rhythm [70], suggesting that these neurons play an important role in fast information processing. Raphe axons innervate the hippocampus and cortex to release serotonin onto target cells [71–73]. Serotonergic afferents have an interesting distribution in the hippocampus, most heavily innervating the border lamina between stratum radiatum and stratum lacunosum moleculare [73]. CCK and calbindin-positive neurons populate this border region, which are potently excited by puff application of serotonin due to the high expression of 5HT₃ receptors, which are ligand-gated ion channels [74]. In an exciting study by Varga and colleagues, channelrhodopsin was introduced into raphe neurons, enabling raphe axons to be excited by blue light [73] (Fig. 6). Activation of these raphe axons evoked an excitatory postsynaptic potential in radiatum interneurons that was both serotonergic and glutamatergic, as revealed by antagonism to both 5HT₃ and glutamate receptors [73]. 5-HT receptors are probably not present on presynaptic terminals of CCK interneurons, as suggested by the failure of 5-HT to alter the frequency or amplitude of miniature inhibitory postsynaptic currents (mIPSCs) [75].

Interestingly, both *in vivo* and *in vitro* data demonstrate that a window of relative quiescence in principal cells follows the raphe-induced excitation of interneurons. This period of quiescence is due to an afterhyperpolarization in pyramidal cells, which is due to disynaptic inhibition from 5HT-3 expressing GABAergic neurons and direct inhibition through 5HT_{1a} receptors [73]. Since this disynaptic inhibition is most likely mediated by CCK-expressing cells, GABA release from these cells should have a prominent asynchronous component [21, 22]. Although the expression of 5HT₃ receptors on CCK interneurons is well characterized, 5HT can elicit a hyperpolarization or no effect in other cell types [33], demonstrating that 5HT can elicit cell-type specific effects in hippocampal interneurons. Unfortunately, little is known about the neurochemical identity of these other interneuron subtypes.

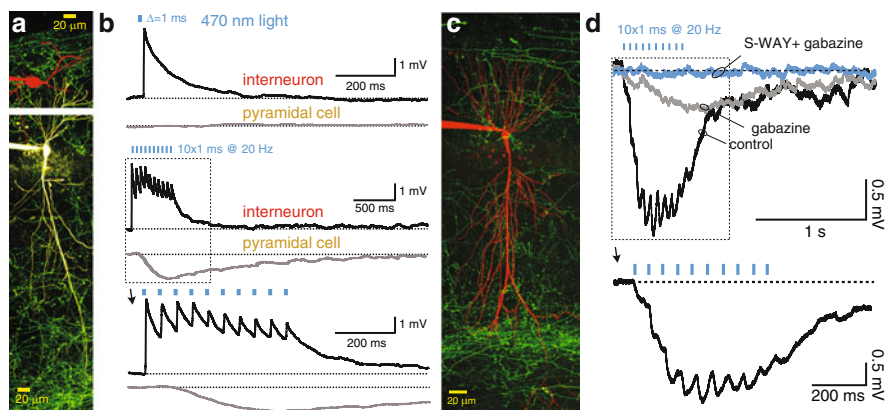


Fig. 6 Raphe fiber activation excites hippocampal interneurons *in vitro*. (a) Image of an interneuron (IN; red) and a CA1 pyramidal cell (PC; yellow-white) recorded sequentially. (b) Averaged responses recorded from the IN (black) and from the PC (gray) to ChR2- photostimulation. Repetitive photostimulation reliably evokes trains of EPSPs in INs but evokes only a slow, small hyperpolarization in CA1 PCs. (c) Image stack of a CA1 pyramidal cell (red) recorded in a dorsal hippocampal slice comprising dense network of eGFP+ fibers. (d) Representative membrane potential traces from CA1 pyramidal cells with pipettes containing low $[Cl^-]$ in intracellular solution in response to repeated photostimulation in control (“control”), in the presence of GABA_A receptor antagonist (gabazine, 20 μ M) and during subsequent application of the 5-HT_{1A} metabotropic serotonin receptor antagonist (“S-WAY”; S-WAY100135, 100 μ M). Note that gabazine blocked fast IPSPs (shown on expanded time scale) and left a slow hyperpolarization, which was blocked by subsequent application of the 5-HT_{1A}R antagonist

3.3 Noradrenergic Control of GABAergic Circuits by the Locus Coeruleus

The axons of locus coeruleus neurons project to all layers of the CA1 hippocampus [76, 77], which often form synaptic specializations with GABAergic interneuron targets [78]. The response of hippocampal interneurons to norepinephrine is heterogeneous [79]. As suggested by laminar specificity in the polarity of its effects, NE appears to have consistent effects on specific classes of interneurons. In virtually all interneurons located in stratum oriens, noradrenaline produces a potent depolarizing action [33, 79–81]. Norepinephrine and the β -adrenergic receptor agonist isoprenaline appear to increase spontaneous firing in O-LM cells through by shifting the activation curve for I_h , the hyperpolarization activated cationic current I_h . In addition, basket cells were reported to depolarize with NE. As some BC cell bodies were located in stratum radiatum, which would be unusual for PV BCs, they are likely to be CCK basket cells [79]. However, other stratum radiatum interneurons exhibit hyperpolarization or reduced excitability to adrenergic receptor activation [33, 79].

Norepinephrine acts on a range of adrenoceptors (AR) with both α and β subtypes localized to both somatodendritic and axonal regions [82, 83]. Recent

studies on neurochemically defined interneuron subtypes indicate that expression of both α [84] and β [85] adrenoceptor subunits differs in a cell type-specific manner. Doze and colleagues employed single cell PCR to examine mRNA transcripts on stratum oriens and stratum radiatum interneurons. Interneurons in stratum oriens were positive for SOM+ and NPY+; in half of these cells, α 1A and/or α 1B AR mRNA [84]. However, in stratum radiatum cells that were CCK+, α 1 was absent in all but one interneuron [84]. The expression of β AR subtypes also appears to be cell type-specific. In a recent paper examining β AR subtypes using immunocytochemistry [85], β 1 ARs were also found in high abundance PV+ cells of stratum pyramidale, which are most likely PV BCs. In contrast, in CCK+ cells, which most likely correspond to CCK BCs and/or Schaeffer-collateral associated interneurons, β 1 ARs were least abundantly expressed whereas β 2 ARs were most abundantly expressed. Finally, CR+ cells, a unique class of interneurons thought to target other interneurons [86, 87], possess neither β 1 nor β 2 ARs [85]. Given that each of these neurochemically defined interneuron classes have distinct intrinsic firing properties, differential expression of α and β AR subtypes suggest that each interneuron subclass could elicit a distinct type of response to adrenergic receptor activation.

3.4 Histaminergic Control of GABAergic Circuits by the Tuberomammillary Nucleus

Histamine is reported to regulate interneuronal excitability as indicated by the dramatic increase in spontaneous inhibitory synaptic potentials in both the dentate gyrus and hippocampus [88, 89]. Interestingly, although histamine enhances ACh release into the hippocampus by exciting cholinergic neurons, direct (via H2) and indirect (via mAChRs) effects of histamine on interneuron excitability do not seem to have equivalent effects. H2 receptor activation has been shown to regulate Kv3.2 channels that mediate the rapid repolarization of the interneuron action potential [90]. However, the shape of the action potential is resistant to mAChR activation in PV BCs [37], suggesting that histaminergic H2 and mAChR M1 receptors couple to different ionic conductances. A more detailed understanding of how histamine modulates GABAergic transmission on a cell type-specific basis remains to be established.

4 Concluding Remarks

The promise of a logical and rational relationship existing between a neuron's anatomical attributes and its physiological functions has inspired over a century of neuroanatomical research. The existence of morphologically and physiologically

distinct interneuron classes raises the possibility that they could be controlled independently of one another. Indeed, in a commentary entitled “Cajal’s rational psychology,” Roger Nicoll presented a hypothesis that different neuromodulatory systems might exert precise control over principal cell excitability by engaging different interneuron subtypes: “Given the rich variety of neurotransmitter fiber systems (noradrenergic, cholinergic, opiodergic, to name just a few) that converge on the hippocampus, it is easy to imagine how different sets of interneurons could be selectively engaged in different behavioral states. In this context, it will be important to define the pharmacological fingerprint of the three classes of interneurons described by Buhl et al.” [91]. Since Nicoll threw down the gauntlet in 1994, the quest to define neuromodulatory specializations, or “pharmacological fingerprints,” of specific subtypes of hippocampal interneurons still remains at an early stage and is complicated by many factors. We now know that a single interneuron subtype can be modulated by more than one neuromodulatory system. Conversely, we know that a single neuromodulatory system can modulate more than one cell type. Differences in the expression of neuromodulatory receptors and target specificity of neuromodulatory afferents complicate the circumstances further. However, when taken as a whole, the above evidence suggests that neuromodulatory systems of the RAS do engage interneuron subtypes in a cell type-specific manner. The routine use of post-hoc immunocytochemistry, single cell PCR, transgenic mice in which neurochemically distinct interneuron subtypes are visualized with GFP, and, ultimately, the selective ablation of neuromodulatory specializations in neurochemically distinct interneuron subtypes will sharpen these distinctions and accelerate the pace in which the neuromodulatory specializations of interneuron subtypes can be identified. Such studies will greatly strengthen our knowledge of how neuromodulatory systems interface with cortical inhibitory networks to optimize information flow during waking states. In regards to neuromodulation of interneurons, Cajal’s rational psychology has a bright future.

Acknowledgments I thank Mathew Jones and Ewa Zarnowska for their comments on an earlier version of the manuscript, David Bonislawski and Jordan Pauli for providing Fig. 3, and members of the Lawrence laboratory for their comments on the manuscript. This work was supported by NCRR NIH 5 P20 RR015583-10.

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Function of GABA_B and ρ -Containing GABA_A Receptors (GABA_C Receptors) in the Regulation of Basic and Higher Integrated Sleep-Waking Processes

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Abstract Inhibitory processes have long been considered crucial for brain functioning. While early studies focused on GABA_A receptor influences, nowadays research has expanded our knowledge of the inhibitory processes that specifically regulate waking and sleep by including GABA_B and the more recently discovered ρ -containing GABA_A (GABA_C) receptors as well. The main results have shown that both GABA_B and GABA_C receptor antagonists increase waking and decrease slow-wave sleep. In contrast, while GABA_B receptor antagonists increase REM sleep, GABA_C receptor antagonists inhibit it.

Keywords Brainstem · Hypothalamus · Thalamus · Cortex · Inhibition · REM sleep

1 Introduction

Insomnia is among the most frequent complaints encountered in a doctor's office. Although often related to anxiety, it generally reflects a failure of the central inhibitory processes. These processes were identified in the brain as early as 1881, when Bubnoff and Heidenhain [1] used cortical stimulation to inhibit both peripheral and cortical stimulation-induced movements. Later, Hess [2] asserted that "the essential mechanism of sleep cannot be explained differently as active inhibition of some functions of the organism." The first identification of inhibitory processes acting at the neuron level was made by Creutzfeld et al. [3]; Evarts [4] subsequently demonstrated their presence during waking-sleep alternation. Finally, Krnjevic et al. [5] were able to inhibit cortical neurons through the administration

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of gamma aminobutyric acid (GABA). Today, GABA is considered to be the main inhibitory transmitter in the brain, with 20% of brain neurons estimated to be GABAergic [6].

The ionotropic GABA_A receptors were the first to be discovered. They are coupled to Cl⁻ channels, and their agonists are used as anxiolytics and hypnotics. Later discoveries revealed the existence of the GABA_B [7] and GABA_C [8] receptors, the influences of which will be reviewed below.

2 Results

2.1 GABA_B Receptors

The metabotropic transmembrane GABA_B receptors “belong to Family 3 of the G-protein-coupled receptors which share the characteristic of a large extracellular amino terminal domain that contains a so-called ‘Venus flytrap’ ligand-binding site” (p 35) [9]. GABA_B receptors are obligatory dimers comprising one GABA_{B1} subunit and one GABA_{B2} subunit. “These are somewhat similar, but the agonist binding site is within the extracellular N terminus only on GABA_{B1}, whilst the coupling to G-proteins is only found on GABA_{B2} which itself has no known agonist” (p 318) [10]. Both subunits are functionally inseparable because mice lacking the GABA_{B1} subunit show a general absence of GABA_B responses [11]. GABA_B receptors can be postsynaptic, where they activate K⁺ channels and cause the hyperpolarization of target neurons, or presynaptic, where they act as autoreceptors and inhibit GABA release. They can also exist as heteroreceptors, inhibiting, for example, glutamate release [12, 13]. In the two latter cases, they reduce Ca²⁺ conductance. GABA’s affinity for GABA_B receptors is lower than it is for GABA_A receptors [14]. Moreover, while GABA_A receptor activation induces a rapid and short inhibition of neurons, GABA_B receptor activation leads to a low, delayed, long-lasting inhibition [15–17]. “The low inhibitory postsynaptic potential (IPSP) would tend to reduce background firing rates, reduce responsiveness to weak excitatory stimuli, but leave responsiveness to strong transient stimuli unimpaired” (p 463) [18].

The influence of GABA_B receptors on sleep-waking stages was first addressed using global approaches, which were later refined in more specific studies to address the various local roles of the receptor.

It was first shown that lethargic mutant (*lh-lh*) mice showed an increased number of GABA_B receptors in the neocortex and the thalamus, while GABA_A receptors were unchanged [19, 20]. In parallel, the blockade of GABA_B receptors in the thalamus decreased electrophysiological activities indicative of deep slow-wave sleep (<10 Hz), while light slow-wave sleep was increased [21]. These results were in accordance with a subsequent study showing that the daytime intraperitoneal (ip) administration of a GABA_B receptor antagonist (CGP 35348) decreased slow-wave

sleep and increased waking [22]. In older rats, however, administration during the active nighttime period increased slow-wave sleep [23]. Regardless of this difference, both these studies showed that the blockade of GABA_B receptors increases rapid eye movement (REM) sleep. In a double-blind study in humans, the GABA_B receptor agonist baclofen was shown to increase slow-wave sleep and decrease REM sleep [24], in agreement with Gauthier et al.'s result [22]. A separate study, however, found the same increase in slow-wave sleep but an increase in REM sleep [25]. Finally, a recent study [26] found that intracerebroventricular (icv) injection of baclofen reversed the waking increase and slow-wave sleep decrease that is normally induced by physical stress; the decrease in REM sleep, however, persisted. In contrast, baclofen did reverse the increased REM sleep normally caused by psychological stress; the other sleep-waking stages remained unaffected [27].

Extending the results obtained in these general studies, more recent work has addressed the influences of GABA_B receptors in detail.

2.1.1 Thalamic and Cortical Electrophysiological Influences of GABA_B Receptors

Different electrocortical patterns can be observed during slow-wave sleep. After falling asleep, synchronized oscillations are seen, with spindles (σ) appearing first (12–14 Hz); in humans, these are characteristic of light sleep stage II. In animals, their frequency varies between 5 and 15 Hz (mean 11.2 Hz) [28]. During deeper sleep, delta waves (δ) (1–4 Hz) appear in stage III and are generalized by stage IV. More recently, slow oscillations (<1 Hz) have been described as well [29].

For more than half a century, it has been recognized that thalamocortical relations are essential for sleep-waking patterns [30, 31]. For example, when the activating brainstem ascending influences that support waking processes are reduced, cortical spindles are recorded in the thalamus [32, 33]. Further, thalamic spindles are observed in neocorticated cats [34, 35]. Moreover, recent research has indicated that the reticular nucleus generates the spindles, as either its disconnection from relay nuclei [36] or its lesion [37] suppresses thalamocortical spindles. Also, the deafferented reticular nucleus generates spindle rhythmicity [38]. When intracellular recordings are processed in relay nuclei neurons, the spindles occur concomitantly to barrages of inhibitory postsynaptic potentials that occur in synchrony with bursts of action potentials in the neurons of the reticular nucleus [39]. “The spindle-related spike bursts of reticular nucleus impose rhythmic IPSPs in target thalamic relay neurons, leading to postinhibitory rebound bursts that are transferred to the cortex.” (p 3293) [40].

The reticular nucleus contains the highest concentration of GABAergic neurons [41] in the brain [17]. Both GABA_A and GABA_B receptors are involved in spindle generation and regulation. “The persistence of normal spindle waves despite strong or complete block of GABA_B receptors. . . indicate that these receptors do not make an essential contribution to the normal generation of spindle waves in thalamic

relay cells” [39] (p 658). In contrast, when GABA_A receptors are blocked, the intrinsic frequency of spindles decreases and spike-and-waves are observed; this pattern is similar to that of absence epilepsy and is inhibited by the cholinergic nucleus basalis stimulation [37]. GABA_B receptor antagonists suppress this abnormal activity, demonstrating the influence of this receptor on the generation of this pathological rhythm. Indeed, GABA_B receptor antagonists are used as a medication for this disease [42–44].

The further sliding from spindling to delta waves during advanced slow-wave sleep is generated by thalamocortical neurons, when their resting membrane potential V_m increases from -60 to -65 mV. These delta waves result from low-threshold cellular spikes followed by afterhyperpolarizing potentials [45]. The spindle-delta wave evolution is related to a “functional reorganization” of the reticular nucleus–thalamocortical neuron relationship, “allowing the same collection of neurons to exhibit different behaviors” (p 15417) [46]. This first occurs through a more pronounced disfacilitation of thalamocortical neurons due to a lowering of ascending activating influences coming from the brainstem. These influences involve, specifically, cholinergic influences mainly originating from the pedunculopontine and lateral dorsal tegmental nuclei [47] and noradrenergic and serotonergic processes [48] originating from the locus coeruleus and raphe nuclei, respectively. Indeed, all of these ascending neurons decrease their firing rates during advanced slow-wave sleep [33, 49–52]. Second, there is a tendency to lower the activation of reticular nucleus neurons by (1) ascending noradrenergic, serotonergic influences; (2) cortical descending glutamatergic disfacilitation [53]; and (3) the lowering of excitatory influences coming from the collaterals of thalamocortical neurons. Underwith the influence of the remaining descending cortical excitatory neurons, the delta oscillations of the thalamocortical neurons are related to a decrease in fast GABA_A hyperpolarizing currents and a maintenance of crucial, slow, long-duration GABA_B hyperpolarizing currents; these are both required for the synchronized functioning of thalamocortical neurons. It is of interest that a significant decrease in normalized cerebral blood flow (rCBF) has been observed in the thalamus during delta and spindling activity [54, 55].

The more recently discovered slow oscillations (<1 Hz) [29] also involve hyperpolarizing processes. These potentials were first identified at the cortical level, more precisely related to pyramidal neuron activity. “They consisted of slow depolarizing envelopes with superimposed full action potentials or presumably dendritic spikes followed by long-lasting hyperpolarizations” [29], separated by long periods of neuronal silence. Although this slow activity is unaffected by thalamocortical neuron destruction, it spreads from the cortex to thalamocortical and reticular neurons. In vitro, GABA_A receptor activation is responsible for the rapid, short-lasting hyperpolarization, whereas GABA_B receptor activation is responsible for the slow, late hyperpolarizing current [18]. In contrast, in vivo, the K^+ conductance is not primarily of GABA_B receptor origin, as GABA_B antagonists do not significantly affect the slow hyperpolarization. Adenosine could induce a similar hyperpolarizing effect. A significant cortical disfacilitation following the slow oscillation seems to be responsible for the hyperpolarization [16]. A recent

study confirmed that the slow, long-lasting hyperpolarization occurring during slow-wave sleep is not related to GABA_B receptor-related currents, but rather mainly due to a “dramatic decrease of synaptic activity” [56].

2.1.2 Specific Structures Involved in the Sleep-Waking Cycle

Brainstem Level

Research on this area was initially devoted to the processes underlying REM sleep. Before the role of GABA in this process was recognized, now old research showed that systemic administration of barbiturates not only induced sleep but inhibited REM sleep [57, 58], replacing it with an extended period of the preceding intermediate stage [57, 59].

The identification of specific GABAergic processes playing a role in REM sleep modulation involved numerous studies, which were carried out prior to the discovery of the particular function of the GABA_B receptor. For example, it was shown that GABA, which is increased during REM sleep in the locus coeruleus [60], favors this sleep stage: GABA_A antagonists prevent the silencing of locus coeruleus neurons, which is required for REM sleep [61], and also significantly reduce REM sleep [62, 63]; in the other direction, GABA infusion increases REM sleep [64]. The GABAergic influences on the locus coeruleus mainly originate in the medulla oblongata prepositus hypoglossi nucleus [65–68]. Similarly, GABA release is increased in the dorsal raphe nucleus, and infusion of the GABA_A agonist muscimol increases this sleep stage [69]. The GABAergic afferents come from the lateral preoptic area and the ventral periaqueductal gray [70, 71]. Finally, several studies have addressed the influence of GABA on REM sleep-on pontine structures [72]. For example, infusion of the GABA_A antagonist bicuculline into the pontine oralis nucleus, in the vicinity of the peri-locus coeruleus (the sublateralodorsal nucleus of rats), induces long-lasting REM sleep episodes and increased waking [73–76]. Further, c-Fos-expressing GABAergic neurons are decreased in association with REM sleep rebound [77, 78]. Similarly, antisense oligonucleotides against glutamic acid decarboxylase (GAD) mRNA increase REM sleep [73]. Consistent with this, the agonist muscimol decreased REM sleep [79]. These latter results suggest an REM sleep-off influence of GABA in this area [80].

Therefore, the influence of GABA in REM sleep-generating processes has become increasingly evident in recent years [76, 81]. However, these results have almost exclusively concerned GABA_A receptor processes. The specific function of GABA_B receptors is only beginning to be elucidated.

The first study addressing GABA_B receptor processes, already mentioned above with respect to GABA_A receptor influences [74], showed that the GABA_B receptor antagonist saclofen, while less efficient than the GABA_A receptor antagonist bicuculline, can induce REM sleep when infused into the REM sleep-on pontine oralis nucleus, in the peri-locus coeruleus α area. This was confirmed soon

afterwards with baclofen infused at the same level, which increased waking and inhibited REM sleep [82]. The same year, it was shown that muscimol (a GABA_A receptor agonist) decreases noradrenaline release and promotes REM sleep when infused into the locus coeruleus, while baclofen has no effect [83, 84]. The absence of GABA_B receptor influence on REM sleep was also shown by baclofen infusion into the forebrain basalis nucleus (Meynert nucleus in humans), which increased slow-wave sleep and decreased waking but did not modify REM sleep; muscimol, on the other hand, inhibited REM sleep [85]. At the pedunculopontine tegmentum level, where there is a high density of cholinergic REM sleep-on neurons, infusion of baclofen suppressed REM sleep in a dose-dependent manner, whereas the GABA_A agonist isoguvacine was without influence [86]; therefore, the REM sleep-on neurons were strongly inhibited by baclofen. Moreover, the other sleep-waking stages were also disturbed, as waking was reduced and slow-wave sleep increased in a dose-dependent manner [86]. It was later shown that this inhibition of REM sleep mediated by GABA_B receptor activation occurs through adenylyl cyclase inhibition, preventing the activation of the intracellular cAMP-dependent protein kinase A (cAMP-PKA). This was demonstrated using Sp-adenosine 3',5'-cyclic monophosphothionate triethylamine (SpCAMPS), an activator of cAMP-PKA [87]. When baclofen was infused by itself into the pedunculopontine area, it again decreased waking, increased slow-wave sleep, and nearly suppressed REM sleep. The simultaneous administration of SpCAMPS, however, partially reversed the effect of baclofen alone on waking and slow-wave sleep, and significantly increased REM sleep. SpCAMPS by itself reinforced the effect of both compounds given together. At odds with this result, but on decerebrated cats, another study showed that baclofen infusion into the pedunculopontine nucleus did not modify REM sleep [88]. This result was in accordance with the observation that the GABA_B antagonist CGP 35348 had no effect on locally induced inhibitory postsynaptic potentials induced in vitro by rubral stimulation [89].

Hypothalamus

The hypothalamus has long been viewed as crucial for sleep-waking processes [90–99]. Recent research has shown that the anterior hypothalamic area (medial and ventrolateral preoptic nuclei) promotes sleep processes through GABA neurons colocalized with galanin. These neurons inhibit posterior hypothalamic histaminergic [100] and orexin [101] neurons, and there are also mutually inhibitory influences with monoaminergic brainstem neurons that support waking [98, 102]. Indeed, ventrolateral [103] and median [104] preoptic neurons show specific c-Fos gene activation during established sleep, and GABA release is increased in the posterior hypothalamus during sleep [105]. Histamine favors waking processes, as classical antihistaminergic drugs induce sleepiness [106, 107] and H₃ autoreceptor antagonists increase waking [108]. Histaminergic neurons, which only fire during waking [109, 110], send terminals particularly to the hypothalamic preoptic nuclei [111].

At the same time, with respect to sleep-waking processes, several recent studies have underlined the major influence of hypocretin/orexin neurons in the posterior hypothalamus perifornical area. Indeed, these neurons project to many anterior and brainstem structures involved in sleep-waking regulation, particularly the locus coeruleus [112], the pontis reticularis oralis nucleus [113], and the preoptic area [114]; they discharge essentially during active waking [115], with occasional spiking during phasic REM sleep [116]. A failure in hypocretin neuron function favors somnolence, cataplexy, and narcolepsy in both animals [117, 118] and humans [119, 120].

GABA_B receptors are involved in both histaminergic and orexin neuron function. The GABAergic inhibition of histaminergic tuberomammillary neurons, as revealed by postsynaptic hyperpolarization induced by preoptic area stimulation, is decreased by baclofen, and this effect is suppressed in turn by the GABA_B antagonist CGP 35348 [121]. Thus, the autoreceptor activation indirectly promotes histamine release and waking-supporting processes. Similarly, GABA_B receptors modulate hypocretin/orexin neuron function. It was first shown on hypothalamic slices that GABA_B receptors are present on hypocretin neurons [122]. Indeed, when lateral hypothalamic neurons were stained with antibodies against the hypocretin-2 peptide and the GABA_{B(1)} subunit, several neurons were immunoreactive for both hypocretin-2 and GABA_{B1} [101]. In the presence of GABA_A receptor blockers, baclofen caused the hyperpolarization of these neurons, and this effect was blocked by the antagonist CGP 52432. Moreover, it was shown by the same authors that GABA_B receptor activation decreases glutamate and GABA release. Consequently, both presynaptic and postsynaptic GABA_B receptors act directly and indirectly on hypocretin neurons. A major study into the influence of GABA_B receptors on sleep-waking processes was performed in knockout mice (oxGKO) lacking the GABA_{B(1)}-binding site [123], i.e., without GABA_B receptor function (see above). In an *in vitro* preparation, whereas baclofen did not hyperpolarize oxGKO hypocretin neurons, it did increase fast spontaneous inhibitory (GABA_A) postsynaptic potentials. This demonstrated that GABA_B receptors situated on GABA_A neuron terminals were suppressed. The results further showed that hypocretin neurons became less sensitive to both local inhibitory and excitatory influences (as well as to serotonergic and CCK ones), and that this absence of modulation was responsible for the observed behavior disturbances in the subsequent *in vivo* study. Indeed, while the overall percentages of sleep-waking stages were unchanged in the knockout mice, there was a severe fragmentation of the duration of the stages. Moreover, the δ wave power during slow-wave sleep was increased (showing facilitated fall into deep slow-wave sleep) and the hippocampal-originating theta wave power was decreased during REM sleep. The impossibility of maintaining stable behavioral states is one characteristic of narcolepsy [124], and the weak GABA_B agonist gamma hydroxybutyrate [125] is a treatment for narcolepsy. Recent results [123] may explain the effect of GABA_B agonists on sleep-waking processes: "They activate postsynaptic GABA_B receptors on hypocretin neurons which results in decreased GABA_A input from local GABAergic (inter)neurons. This, in turn, may increase sensitivity of hypocretin neurones to inhibitory GABA_A

input from, for example, sleep-active neurons in the preoptic area” [123]. The authors concluded that the fragmentation of the sleep-waking cycle that often occurs in the elderly could be consecutive to a failure of the control of hypocretin neurons by GABA.

In the distinct but related field of biological rhythm regulation, it has been shown in experiments measuring wheel running activity that GABA_B receptor activation by baclofen infusion into the suprachiasmatic nucleus dose-dependently reduces the effects of both phase-delaying and phase-advancing responses to light [126]. Baclofen suppresses GABA transmission in this nucleus, an effect that can be suppressed by the antagonist saclofen. There was no baclofen-induced postsynaptic hyperpolarization, meaning that its effect was presynaptic; there are GABA auto-receptors on GABA afferent terminals [127]. This suprachiasmatic infusion of baclofen during the daytime did not modify free running activity in diurnal and nocturnal rodents; however, night administration significantly decreased the ability of light to induce phase delays in diurnal rodents [128].

Our increasing knowledge of brain GABA processes highlights the crucial importance of GABA_B receptors for basic neurophysiological activities, as well as for more integrated waking and sleep behavior. However, as addressed in the next section, higher integrated processes are also regulated by GABA_B receptor function.

High Integrated Processes

As indicated above, GABA_B receptor modulation can suppress absence epilepsy symptoms, which mainly occur during nonattentive waking and drowsiness. Indeed, established results have shown that GABA_B antagonists improve absence epilepsy [44, 129].

Moreover, although it is sometimes difficult to distinguish physiological from psychological dependency in drug addiction, with its associated sleep disturbances [130], baclofen promotes abstinence: “It decreases cocaine self-administration in rats and reduces cocaine craving and intake in individuals with a history of cocaine dependence” (p 325) [10]. At the same time, however, baclofen can cause certain negative secondary disturbances (hypothermia, muscle relaxation), and new positive modulators of the GABA_B receptor-like CGP 5633A or GS39783 [131] are better indications for addiction treatment.

For waking cognitive processes, GABA_B receptor antagonists promote waking memory processes in animals. For example, CGP 36742 improves passive avoidance tests in mice, facilitates partner recollection in rats, and improves conditioned spatial color tasks in monkeys [43, 132]. Moreover, CGP 55845 reverses age-related learning impairment (olfactory discrimination learning) [133], and there is an induced long-term improvement in other memory tests [134]. Finally, knockout mice lacking pre- and postsynaptic GABA_{B(1)} receptors show impaired memory [11].

GABA_B receptor activity has also been shown to be involved in anxiety [135] and depression [136, 137], where there is pressure for REM sleep occurrence in

patients as well as in trouble-free relatives [138, 139]. Knockout mice lacking GABA_B receptor function show increased anxiety, which can be reversed by the positive modulator GS39783 [140]. Thus, GABA_B positive modulators now represent candidates for the treatment of depression. It was further shown that the positive effect of CGP 56633A in the swim test can be suppressed by *p*-chlorophenylalanine (PCPA), an inhibitor of tryptophan hydroxylase [141], which suppresses slow-wave sleep [142–145]. Consequently, the influence of GABA_B is mediated by the serotonergic system. Further, the relationship between GABA and serotonin is reinforced by the finding that the cortical GABA level is increased by serotonin-selective reuptake inhibitors [146].

It seems that the most advanced hypothesis concerning the involvement of GABA_B receptors in pathological integrated processes, however, concerns schizophrenia, during which sleep-waking processes are disturbed. Indeed, in schizophrenics, there is a decreased latency of REM sleep occurrence [147–149], an absence of rebound after REM sleep-specific deprivation [150, 151], and a deficit of brain interconnections as shown by gamma rhythm abnormalities [152–155] as in REM sleep [156–158]. Moreover, the same deactivation of the dorsolateral prefrontal cortex occurs during REM sleep [159, 160] and in schizophrenia [161–163].

Several lines of evidence indicate that there is a deficit of cortical inhibitory processes in this disease [164–166]. First, there are fewer GABAergic interneurons in several cortical areas, particularly in the cingulate and prefrontal cortex. Indeed, the interneurons (most probably GABAergic) are reduced in number, particularly in the prefrontal layer II and to a lower extent in layer I. In contrast, pyramidal neurons are at a higher density in layer V [167]. Moreover, mRNA-encoding glutamic acid decarboxylase (GAD), the enzyme responsible for GABA synthesis, is decreased in all layers of the dorsolateral prefrontal cortex [168], and the synthesis and reuptake of GABA are reduced in a subset of dorsolateral prefrontal cortex neurons [169]. In addition, sensory gate control is disturbed in schizophrenics, as responsiveness recovery cycle studies have revealed failures in the inhibition of both the P₅ [170] and N₁₀₀ [171] components of the auditory-evoked potential. The same deficit in underlying N₁₀₀ inhibitory processes has been observed during REM sleep [171]. Also, suprathreshold transcranial magnetic stimulation of the motor cortex induces a large motor-evoked potential, which is followed by the suppression of electromyographic activity, a silence, which is an index of cortical inhibitory processes. This “cortical silent period” (CSP) is increased by baclofen and by the atypical neuroleptic clozapine [172]. In contrast, the short interval cortical inhibition (SICI), which concerns the short-latency response to a test stimulus following a conditioned stimulus, is potentiated by benzodiazepine and reflects a GABA_A receptor process [173]. Some studies have observed clinical improvement when baclofen is added to neuroleptics ([174]; ref in [172]). Although “there is virtually no molecular evidence linking clozapine to GABA_B receptor-mediated inhibitory transmission. . . preliminary evidence does indeed, suggest such link . . . (However) more evidence is necessary for a better understanding of clozapine’s possible direct molecular effects on the GABA_B receptor and the extent to which these mechanisms

translate to treatment in schizophrenia” [172]. Finally, GABA_B receptors situated on dopaminergic terminals inhibit dopamine release, and GABA_B receptor deficits result in a sustained hyperdopaminergic state [175]; this has long been viewed as a main characteristic of schizophrenia [176] and REM sleep in nucleus accumbens [177, 178].

Thus, although for decades most major studies have concerned GABA_A receptor function, GABA_B receptors increasingly appear to be involved in the functioning of basic and higher integrated sleep-waking brain processes.

2.2 GABA_C Receptors

The subclass of GABA_A receptors containing ρ -subunits was first observed at the retinal level and termed GABA_C receptor due to its distinct ligand-binding profile [179, 180]. However, like GABA_A receptors, ionotropic GABA_C receptors are GABA-gated chloride channels [181, 182]. The term GABA_C receptor is used here as abbreviation for ρ -containing GABA_A receptors and does not indicate a separate ionotropic receptor subfamily. The receptors were initially described as comprising three subunits (ρ 1, ρ 2, and ρ 3) [183], but subsequent work revealed the presence of five (ρ 4 and ρ 5) [184]. They are insensitive to bicuculline, barbiturates, and benzodiazepines, thus distinguishing them from GABA_A receptors, and they are also insensitive to baclofen and phaclofen, which typically act on GABA_B receptors [184]. The GABA_C receptors are more sensitive to GABA than are GABA_A and GABA_B receptors. Their channel opening time and their desensitization are also longer than in the other receptors [14, 180–182, 185].

GABA_C receptors are found either alone or colocalized with GABA_A receptors [186], particularly in 10–15% of cases where they are present at postsynaptic sites in the midbrain and the brainstem [187]. The GABA_C ρ 1 subunit is often assembled with the α 1 and γ 2-binding sites of the GABA_A receptor, and the GABA_A receptor modulator zolpidem enhances the GABA_C receptor agonist (*cis*-4-aminocrotonic acid, CACA) response [188]. Accordingly, GABA_A and GABA_C receptor subunits can form heteromeric hybrid receptors in the rat brainstem [188].

In young chicks, injecting the GABA_C receptor antagonists TPMPA and P4MPA into the multimodal association area of the forebrain enhanced short-term memory in a bead discrimination task [189], and intraperitoneal administration of CGP36742, another antagonist, promoted learning and memory in rats subjected to the Morris water maze task [184]. However, injection in the lateral amygdala nucleus of very low doses of muscimol (acting as GABA_C receptor agonist) enhanced, and TPMPA impaired, fear learning and memory in a Pavlovian conditioning experiment [190].

Only few studies have been performed to investigate GABA_C receptors influence on sleep-waking processes. The first such study showed that TPMPA, administered icv in rats, increases waking by enhancing both active and quiet

waking, decreases slow-wave sleep, and decreases REM sleep [191]. In contrast, in another study also performed in rats, the infusion of the agonist CACA in the pedunculo-pontine tegmentum did not modify sleep-waking levels [86]. Finally, the intraperitoneal injection in rats of CGP 36742, an antagonist of both the GABA_B and GABA_C receptors, increased the waking state (particularly quiet waking), decreased slow-wave sleep, and left REM sleep unchanged. It is likely that the increase in REM sleep caused by GABA_B antagonists [22, 23] and the decrease in REM sleep caused by GABA_C receptor antagonists [191] neutralized each other.

Since GABA_C receptors are encountered in various brain structures, particularly in the neocortex [192], it is possible that future research on this receptor will not only increase our knowledge of basic sleep-waking processes but also address its role in the modulation of higher integrated processes related to sleep-waking stages.

3 Conclusion

Inhibitory processes are essential for brain function, as they primarily regulate excitatory neuron processes. Acetylcholine, dopamine, noradrenaline, and serotonin, aside from their central activating influences, have major inhibitory influences, particularly at the cortical level; they also increase the efficiency of neurons by enhancing the signal-to-noise ratio of incoming neuronal information (for ref see [193, 194]). GABA, on the other hand, has only true inhibitory influences, except in the first days of life [195]. Whereas GABA_A receptor influences have been studied for decades and are beginning to be well understood, research on the GABA_B and GABA_C receptors is more recent. The crucial regulatory functions of GABA_B have become increasingly evident, whereas GABA_C receptor studies are only beginning. However, the influence of both receptors on basic sleep-waking behavior in rodents seems to be established. GABA_C receptors show a lower threshold of activation than GABA_A and GABA_B receptors, an essential property that is rich in promise for future clinical applications, when agonists and antagonists will be available for use in humans.

Acknowledgments I thank Dr. Peter Follette for his help with the English.

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Interactions Between GABAergic and Serotonergic Processes in the Dorsal Raphe Nucleus in the Control of REM Sleep and Wakefulness

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Abstract Based on electrophysiological, neurochemical, genetic, and neuropharmacological approaches, it is currently accepted that serotonin (5-HT) functions to promote wakefulness (W) and to inhibit rapid-eye-movement sleep (REMS).

The dorsal raphe nucleus (DRN) has been subdivided into several clusters on the basis of differences in cellular morphology, expression of other neurotransmitters, and afferent and efferent connections. These differences among subpopulations of 5-HT neurons may have important implications for neural mechanisms underlying 5-HT modulation of sleep and wakefulness. The DRN contains 5-HT and non5-HT neurons. The latter express a variety of substances including γ -aminobutyric acid (GABA). The serotonergic cells are present throughout the rostral–caudal extent of the DRN, in all subdivisions of the nucleus. However, they predominate along the midline of the rostral, ventral, and dorsal subdivisions of the raphe nucleus and fire broad spikes with low frequency and a high regular pattern. The activity of DRN serotonergic neurons is greatest during W; it diminishes during slow-wave sleep and is virtually suppressed when the animal starts REMS. GABAergic neurons are abundant throughout the DRN. Although most of the DRN subdivisions contain GABAergic interneurons, they predominate in the lateral wings of the raphe nucleus. All these neurons have significantly faster rates and narrower spike widths than the 5-HT-containing cells.

Local microinjection of 5-HT_{1B}, 5-HT_{2A/2C}, and 5-HT₇ receptor agonists into the DRN selectively suppresses REMS in the rat. On the other hand, local administration of the GABA_A receptor agonist muscimol into the neuroanatomical structure increases REMS in laboratory animals. The inhibition of GABAergic interneurons that express 5-HT_{1B} receptor and the activation of long-projection GABAergic interneurons that express 5-HT_{2A/2C} or 5-HT₇ receptors could be tentatively responsible for the inhibition of cholinergic cells of the laterodorsal and pedunculopontine

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tegmental nuclei and the suppression of REMS. In contrast, the direct inhibition of DRN 5-HT neurons by muscimol tends to facilitate the occurrence of REMS.

It has been established that inhibitory and facilitatory neurotransmitter systems project to the DRN and regulate the activity of 5-HT neurons during the sleep–wake cycle. Among the former is the GABAergic system that can promote REMS by deactivating 5-HT neurons. The finding that direct microinjection of 5-HT_{1B}, 5-HT_{2A/2C}, and 5-HT₇ receptor ligands into the DRN interferes with the occurrence of REMS points to the existence of a supplemental mechanism in the control of 5-HT neurons functional activity.

1 Introduction

The neural structures involved in the promotion of wakefulness (W) are situated in the brain stem, the hypothalamus, and the basal forebrain. The nuclei found in the brain stem include the dorsal raphe nucleus (DRN) and the median raphe nucleus (MRN), which contain serotonergic cells and send long ascending projections to the thalamus, cerebral cortex, and BFB and descending projections to the spinal cord [1] (Table 1).

Neurons in the preoptic area, anterior hypothalamus, and adjacent basal forebrain constitute the sleep-inducing system [2]. A majority of these neurons contain γ -aminobutyric acid (GABA), galanin and melanin-concentrating hormone, three inhibitory neurotransmitters, and project to the basal forebrain and to hypothalamic and brain stem areas involved in the promotion of W, including the DRN [3, 4].

Cholinergic neurons of the laterodorsal and pedunculopontine tegmental nuclei (LDT/PPT) act to promote rapid-eye-movement sleep (REMS). All these neurons are inhibited by, among others, serotonin (5-HT)-containing cells [5, 6]. The REMS induction region of the medial pontine reticular formation (mPRF), one of the neuroanatomical structures proposed to be responsible for REMS generation, includes predominantly glutamatergic neurons that are in turn activated by efferent connections of the LDT/PPT [7].

2 The Structure of the Dorsal Raphe Nucleus

The DRN contains 5-HT and non5-HT neurons. The latter express a variety of substances including GABA, glutamate, and dopamine. In addition, nitric oxide and a number of neuropeptides have been characterized in the DRN. Moreover, numerous brain areas have neurons that project to the DRN and express aminoacids (GABA, glutamate), monoamines (norepinephrine, histamine), acetylcholine, or

Table 1 Afferent and efferent connections of the dorsal raphe nucleus of the rat

Afferent connections	Efferent connections
<i>Telencephalon</i>	
<i>Cerebral cortex</i>	
Frontal, cingulate, orbital, and insular cortices	Frontal, piriform, insular, occipital, entorhinal, cingulate, and infralimbic cortices
<i>Limbic system</i>	
Amygdala (central, anterior, medial, and basolateral nuclei), medial and lateral septal nuclei	Ventral hippocampus, lateral septal nucleus, amygdala, (central, lateral, and basolateral nuclei)
<i>Basal forebrain</i>	
Accumbens nuclei, ventral pallidum, bed nucleus of the stria terminalis, diagonal band	Diagonal band (horizontal and vertical limb)
<i>Neostriatum</i>	
	Posteromedial regions of the striatum
<i>Diencephalon</i>	
<i>Thalamus</i>	
	Midline, intralaminar, and anterior nuclei
<i>Hypothalamus</i>	
Medial and lateral preoptic areas; anterior, lateral and posterior hypothalamic areas; dorsomedial and ventromedial nuclei; tuberomammillary nucleus	Preoptic area, lateral hypothalamic area, supramammillary nucleus
<i>Mesencephalon</i>	
Substantia nigra-pars reticulata, interpeduncular nucleus, central gray	Interpeduncular nucleus, substantia nigra-pars compacta, ventral tegmental area, central gray, mesencephalic reticular formation, pedunculopontine tegmental nucleus, laterodorsal tegmental nucleus
<i>Rhombencephalon</i>	
Raphe nuclei, locus coeruleus, pontine reticular formation	Pontine reticular formation, nucleus reticularis pontis oralis, nucleus reticularis pontis caudalis, nucleus reticularis gigantocellularis, locus coeruleus, median raphe nucleus, nucleus raphe pontis

neuropeptides that directly or indirectly, through local circuits, regulate the activity of 5-HT cells [8–12].

Based on cellular morphology, expression of other neurotransmitters, afferent and efferent connections, and functional properties, 5-HT neurons of the DRN have been grouped into several subnuclei [13, 14]. These differences among subpopulations of 5-HT neurons may have important implications for neural mechanisms underlying 5-HT modulation of sleep and wakefulness.

Lowry et al. [11] have proposed six subdivisions of the DRN that comprise the rostral, ventral, dorsal, lateral, caudal, and interfascicular clusters.

It has been estimated that two-third of neurons found in the DRN of adult rats are serotonergic. The serotonergic cells are present throughout the rostral–caudal extent of the DRN, in all subdivisions of the nucleus. However, they

predominate along the midline of the rostral, ventral, and dorsal subdivisions of the DRN [15].

GABAergic neurons are abundant throughout the DRN. Although most of the DRN subnuclei contain GABAergic interneurons, they predominate in the lateral wings of the raphe nucleus [16, 17]. In addition, GABAergic neurons located in several neuroanatomical structures including the ventral pallidum, the hypothalamus, the mesencephalon, and the rhombencephalon project to the DRN [18]. A number of classes of GABA receptors such as the GABA_A, GABA_B, and GABA_C receptor have been characterized in the central nervous system of several species. The GABA_A receptor, an ionotropic chloride channel, has a predominant role in the modulation of sleep variables. The GABA_A receptor is expressed by GABAergic interneurons located in the DRN. The GABA_B receptor is a metabotropic G-protein-coupled receptor. Its activation enhances 5-HT output in the DRN of the rat [19]. The relevance of the GABA_C receptor in sleep mechanisms is presently unknown.

3 The Functional Activity of DRN Neurons During the Sleep–Wake Cycle

Kirby et al. [20] characterized the electrophysiological properties of 5-HT and non5-HT cells located predominantly in the ventral and interfascicular clusters of DRN slice preparations of the rat. 5-HT neurons showed a significantly lower firing rate, a shorter time constant, and a slower onset of the afterhyperpolarization than non5-HT cells.

Allers and Sharp [21] successfully identified the neurochemical and morphological properties of 5-HT-containing cells and GABA-containing cells of the DRN in urethane-anesthetized rats. The study authors characterized a group of neurons immunoreactive for 5-HT or both 5-HT and tryptophan hydroxylase that fired broad spikes with low frequency and a highly regular pattern. Systemic administration of the 5-HT_{1A} receptor agonist 8-OHDPAT inhibited the firing of these neurons. A fast firing population of cells was described, in addition, which was immunoreactive for glutamic acid decarboxylase and immunonegative for tryptophan hydroxylase. All these neurons had significantly faster firing rates and narrower spike widths than the 5-HT-containing cells.

Electrophysiological recordings in unanesthetized animals allowed McGinty and Harper [22] and Trulson and Jacobs [23] to characterize the activity of DRN 5-HT neurons across the sleep–wake cycle. During quiet waking, 5-HT neurons fire in a slow and regular fashion. During active waking, the neuronal activity shows a 30–50% increase. As the animal enters slow-wave sleep (SWS), the firing rate decreases to 50% of the quiet waking state and is no longer regular. Finally, during REMS, there is a further decrease or even a cessation of neuronal activity.

4 Operational Characteristics of the 5-HT Receptors

The 5-HT receptors can be classified into seven classes designated 5-HT₁₋₇. The 5-HT_{1A} receptor is located on the soma and the dendrites (somatodendritic autoreceptor) of 5-HT neurons and at postsynaptic sites (outside the DRN). In addition, the 5-HT_{1A} receptor has been found to be expressed by non5-HT cells of the DRN [17]. In this respect, 40–50% of immunohistochemically identified non5-HT neurons of the DRN express the 5-HT_{1A} receptor [20].

The 5-HT_{1A} receptor couples to Gi/o, with Gi activation leading to an inhibition of adenylate cyclase. Stimulation of the somatodendritic 5-HT_{1A} receptor inhibits the firing of serotonergic neurons, whereas activation of the postsynaptic receptor induces inhibitory responses on target structures. Activation of postsynaptic 5-HT_{1A} receptor expressed by GABAergic interneurons located in the DRN is expected to inhibit the release of GABA and to indirectly facilitate the activity of 5-HT neurons during W.

The 5-HT_{1B} receptor is linked to the inhibition of adenylate cyclase and is located at presynaptic (5-HT axon terminals) and postsynaptic (outside the boundaries of the DRN) sites. The 5-HT_{1B} receptor has been characterized also in the ventromedial DRN where it is expressed by non5-HT cells [24]. The activation of 5-HT_{1B} receptor expressed by GABAergic interneurons located in the DRN would also inhibit the release of GABA.

The 5-HT_{2A} and the 5-HT_{2C} receptors have striking amino acid homology. They are primarily coupled to Gq, and their actions are mediated by the activation of phospholipase C, with a resulting depolarization of the host cell [25]. Receptors of the 5-HT₂ subfamily are located within postsynaptic structures, predominantly on proximal and distal dendritic shafts. 5-HT neurons of the DRN do not express 5-HT_{2A} or 5-HT_{2C} receptors. The serotonin 5-HT_{2A} and 5-HT_{2C} receptor-containing neurons are predominantly GABAergic interneurons and projection neurons [26]. Thus, activation of 5-HT_{2A} and 5-HT_{2C} receptors expressed by GABAergic cells located in the DRN would result in the decrease of 5-HT neurons firing rate.

The 5-HT₃ receptor is not coupled to G proteins. It directly activates a 5-HT-gated cation channel, which leads to the depolarization of a variety of cells. As a result, there is an increase in the release of GABA, glutamate, norepinephrine, dopamine, acetylcholine, and 5-HT at central sites [27]. At the DRN level, the 5-HT₃ receptor is expressed, among others, by glutamatergic interneurons [28].

The 5-HT₇ receptor is positively coupled to adenylate cyclase via G_s-proteins. 5-HT₇ receptors in the DRN are not localized to serotonergic neurons and consequently do not subserve an autoreceptor function [29]. In this respect, Roberts et al. [30, 31] and Glass et al. [32] have proposed, on the basis of a series of functional studies, that 5-HT₇ receptors in the DRN are localized, at least in part, to GABAergic cells and terminals. The activation of 5-HT₇ receptor expressed by GABAergic interneurons would result in the inhibition of 5-HT-containing cells.

5 Serotonergic and GABAergic Neurons of the DRN and Their Involvement in the Regulation of Sleep and Wakefulness

A series of studies involving the microinjection of 5-HT_{1B}–, 5-HT_{2A/2C}–, and 5-HT₇ receptor agonists into the DRN point to the involvement of GABAergic interneurons and the projection neurons in their effects on REMS.

6 Local Administration of 5-HT Receptor Ligands into the DRN

6.1 Microinjection of 5-HT_{1B} Receptor Ligands

In one study, microinjection of the selective 5-HT_{1B} receptor agonist CP-94253 into the DRN during the light phase caused a reduction of REMS and of the mean duration of REM periods. Pretreatment with the 5-HT_{1B} receptor antagonist SB 224–289 antagonized the CP-94253-induced decrease of REMS. Administration of the GABA_A receptor agonist muscimol prevented also the effect of CP-94253 on REMS [33]. Activation of the 5-HT_{1B} receptor in the DRN has been shown to inhibit the release of GABA [34]. Thus, the decrease of GABA inhibitory tone on 5-HT neurons would result in an increment of 5-HT release at postsynaptic sites and the suppression of REMS. This conclusion is drawn from the fact that microinjection of muscimol prevented the CP-94253-induced decrease of REMS.

6.2 Microinjection of 5-HT_{2A/2C} Receptor Ligands

In another study, infusion of the 5-HT_{2A/2C} receptor agonist DOI into the DRN induced a significant reduction of REMS and of the number of REM periods in the rat. Pretreatment with the selective 5-HT_{2A} or 5-HT_{2C} receptor antagonists EMD 281014 or SB-243213, respectively, prevented the DOI-induced suppression of REMS, which indicates that it was mediated by the 5-HT_{2A} and 5-HT_{2C} receptors located in the DRN [35]. 5-HT neurons of the DRN do not express 5-HT_{2A} or 5-HT_{2C} receptors. The serotonin 5-HT_{2A} and 5-HT_{2C} receptor-containing neurons are predominantly GABAergic interneurons [36]. Systemic or intrapaphe administration of DOI inhibits the firing of serotonergic neurons in the DRN and reduces the extracellular concentration of 5-HT [37]. The reduction of the firing rate of 5-HT neurons in the DRN after systemic administration of DOI is reversed by the 5-HT_{2A} receptor antagonist MDL 100907 or the GABA_A antagonist picrotoxinin [38]. Thus, REMS suppression after DOI microinjection into the DRN is not related to

the inhibition of 5-HT neurons functional activity. Many GABAergic interneurons in the DRN contribute to long projections that reach the LDT/PPT [39]. However, the role of this additional circuitry in the regulation of sleep is still a matter of debate. It could be tentatively proposed that activation of long-projection GABAergic neurons by DOI inhibits the activity of cholinergic cells in the LDT/PPT and decreases REMS.

Interestingly, Amici et al. [40] locally microinjected DOI or the 5-HT₂ receptor antagonist ketanserin into the LDT of rats. DOI significantly decreased the number of REMS episodes, whereas ketanserin induced the opposite effect. The finding by Fay and Kubin [41] that 5-HT_{2A/2C} receptors are located not on cholinergic cells but on GABAergic interneurons intermingled with mesopontine cholinergic cells tends to explain the inhibitory effect of DOI on cholinergic LDT neurons and the reduction of REMS episodes.

6.3 Microinjection of 5-HT₇ Receptor Ligands

Microinjection of the 5-HT₇ receptor agonist LP-44 into the DRN during the light period caused a reduction of REMS and of the number of REM periods in the rat. Pretreatment with the 5-HT₇ receptor antagonist SB-269970 prevented the LP-44-induced suppression of REMS [42]. Roberts et al. [30] and Glass et al. [32] have proposed, on the basis of a series of functional studies, that 5-HT₇ receptors in the DRN are expressed mainly by GABAergic cells and terminals. The activation by LP-44 of 5-HT₇ receptors expressed by projection GABAergic neurons would be tentatively responsible for the inhibition of REM-on cholinergic cells and the suppression of REMS.

7 GABAergic Inhibitory Afferents to the DRN

In addition to the GABAergic interneurons that have been characterized within the boundaries of the DRN, GABAergic cells located in several neuroanatomical structures including the basal forebrain, the hypothalamus, the mesencephalon, and the rhombencephalon project to the raphe nucleus.

GABA has been made responsible for the reduction of the activity of 5-HT neurons during SWS and REMS. Accordingly, iontophoretic application of GABA decreases the activity of DRN 5-HT neurons and this effect is antagonized by the GABA_A receptor antagonist bicuculline [43]. Furthermore, Nitz and Siegel [44] have found that REMS is accompanied by a significant increase of GABA release in the DRN and that local administration of the GABA_A receptor agonist muscimol into the raphe nucleus increases REMS, whereas picrotoxin blocks its occurrence.

According to Luppi et al. [45], the GABAergic inhibition involves post and presynaptic mechanisms. Thus, the reduction of the functional activity of 5-HT

neurons would depend not only on the postsynaptic GABAergic input but also on the GABAergic inhibition of excitatory noradrenergic, histaminergic, cholinergic, and orexinergic inputs to 5-HT cells.

However, the role of GABA in the inhibition of 5-HT cells during sleep is not accepted unanimously. In this respect, it has been argued that the reduced level of spontaneous firing of DRN 5-HT cells is caused exclusively by disfacilitation due to the functional removal of tonic noradrenergic, histaminergic, and orexinergic inputs to these neurons [46].

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GABA-ergic Modulation of Pontine Cholinergic and Noradrenergic Neurons for REM Sleep Generation

Dinesh Pal and Birendra Nath Mallick

Abstract The mechanism of generation of rapid eye movement sleep (REMS) has been intensely studied since its discovery in cat and rat models. Since then, the neuropharmacological as well as electrophysiological studies have brought out the prominent role played by the noradrenergic (NA-ergic) neurons in locus coeruleus (LC) and cholinergic neurons in laterodorsal/pedunculopontine tegmentum (LDT/PPT) in REMS generation. The NA-ergic neurons cease firing during REMS, and are known as REM-OFF neurons, whereas cholinergic neurons increase their firing rate during REMS, and are known as REM-ON neurons. The interaction between NA-ergic LC neurons and the cholinergic LDT/PPT neurons has long been postulated as a central mechanism of REMS generation. Interestingly, LC and LDT/PPT have a large number of GABA-ergic neurons and also receive GABA-ergic afferents from other brain areas. Studies from our as well as other sleep laboratories have provided the evidence indicating that GABA plays a critical neuromodulatory role in the interaction between pontine NA-ergic and cholinergic systems. On the basis of the studies done in our laboratory, it was proposed that the cholinergic projections from LDT/PPT into LC excite GABA-ergic neurons to release GABA, which inhibit the LC NA-ergic neurons to increase REMS. In PPT, GABA acts on the NA-ergic axonal terminals from LC to inhibit the release of noradrenaline (NA), thereby disinhibiting the cholinergic REM-ON neurons and increasing REMS. Further, we have shown that the increase in intracerebral NA as a consequence of noncessation of LC NA-ergic neurons (as during REMS deprivation) increases Na–K ATPase activity. The increased Na–K ATPase activity affects the membrane excitability,

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which along with physiological/morphological changes induced by elevated NA may contribute to the pathology associated with REMS related disorders.

Keywords Agonist and antagonist of adrenergic · Cholinergic · GABAergic-receptors · Laterodorsal tegmentum · Locus ceruleus · REM OFF and REM ON neurons

1 Introduction

Rapid eye movement sleep (REMS) is an endogenous ultradian rhythm found in most mammalian and avian species studied so far [1]. It was first reported in humans by Aserinsky and Kleitman [2] and soon after that, Dement [3] reported the presence of a similar phenomenon in cats. REMS is characterized by low voltage, high frequency cortical electroencephalogram with a predominance of theta waves, bursts of rapid eye movements, and muscle atonia in antigravity muscles. Physiologically, it is marked by arrhythmic changes in respiration and heart rate, phasic muscle twitches, elevated brain temperature and metabolic rate, as well as increased sympathetic tone [4]. There is a broad consensus that REMS is actively generated by the interaction between distinct neurons located within pontine nuclei in the brainstem [5–7]. Besides the studies involving the loss/gain of function (through electrical/pharmacological lesion/stimulation), direct neural recordings from pontine nuclei have contributed immensely to our understanding of the physiology of REMS. Extracellular recordings from pontine nuclei in freely moving, normally behaving animals have identified distinct populations of neurons that (1) increase discharge before the onset of REMS and continue for the duration of the state, known as REM-ON neurons, and (2) decrease discharge before the onset of REMS and remain suppressed for the duration of the state, known as REM-OFF neurons.

The REM-ON neurons have been recorded most notably from laterodorsal/pedunculopontine tegmentum (LDT/PPT) [8, 9], whereas the REM-OFF neurons have been reported from locus coeruleus (LC) [10, 11] and dorsal raphe (DR) [12]. The LC has the A-6 group of noradrenalin (NA)-ergic neurons, which innervate and supply most of the NA to the entire brain [13]. The LDT/PPT has a prominent cholinergic population, which sends projections to the thalamus and pontine reticular formation (PRF) [13, 14]. LC and LDT/PPT have reciprocal anatomical projections [15], and there is a large body of evidence showing that pontine aminergic and cholinergic systems interact with each other for REMS generation [6]. The REM-OFF neurons are active all through except during REMS, whereas the REM-ON neurons remain inhibited all through except during the REMS. Therefore, it is evident that inhibitory mechanisms constitute an important component of the neural circuitry for REMS generation/regulation. In this chapter, we will elaborate on the inhibitory GABA-ergic modulation of pontine

noradrenergic (LC) and cholinergic (LDT/PPT) neuronal systems in the context of recent developments to bring out the key role played by them in REMS regulation.

2 Noradrenergic Regulation of REMS

There is a long history of pharmacological manipulations aimed at LC NA-ergic system to study the role of NA in REMS regulation. The early and recent studies involving lesion of LC as well as systemic administration of NA-ergic agonists and antagonists showed an association between NA and REMS with differential roles for $\alpha 1$, $\alpha 2$ and β - NA-ergic receptors [16–18]. Extracellular recording from LC of cats and rats provided strong evidence for the involvement of NA in REMS regulation. The LC NA-ergic neurons show a state-dependent discharge across sleep–wake states and have been classified as REM-OFF neurons. REM-OFF neurons show highest discharge rates during wake state, progressively decreases with the onset of non-REMS, falling almost silent during REMS [10, 11]. In contrast, during REMS deprivation (REMSD), the LC REM-OFF neurons do not cease but continue firing [19].

Reversible inactivation of LC neurons through (1) localized cooling [20], (2) GABA microinjection into LC [21], or (3) by an increase in GABA concentration in LC through stimulation of GABA-ergic prepositus hypoglossus (PrH), increases REMS [22]. In addition to the GABA from pontine interneurons and PrH, GABA-ergic/Galanin-ergic neurons in ventrolateral preoptic (VLPO) and extended VLPO area have also been shown to be involved in REMS generation, possibly through inhibition of LC neurons [23, 24]. Activation of LC neurons by (1) continuous, mild (low intensity, low frequency) electrical stimulation [25], (2) withdrawal of GABA-ergic inhibition through infusion of GABA-A antagonist into the LC [26], or (3) by infusion of orexin into the LC decreased the time spent in REMS [27]. LC receives orexinergic projections from the neurons localized in perifornical hypothalamic area [28]. Disinhibition of perifornical hypothalamic neurons activates LC NA-ergic neurons [29] and pharmacological stimulation of perifornical neurons through glutamate microinfusion decreased REMS [30]. Thus, the activation of LC NA-ergic neurons strongly correlates with the inhibition of REMS whereas inactivation of LC NA-ergic neurons shows a correlation with the promotion/generation of REMS. As a corollary, it can be argued that the continuous activity or noncessation of the NA-ergic LC-neurons, as was observed during REMSD [19], is likely to maintain higher systemic and intracerebral NA levels during and following REMSD. Indeed, REMSD increases the levels of NA in serum [31] as well as in the brain [32]. The activity of tyrosine hydroxylase (TH), the first rate-limiting step enzyme for the synthesis of NA, shows increased expression in the brain during REMSD [32–34]. As opposed to TH, the activity of NA degrading enzyme (monoamine oxidase-A) decreases in the brain after REMSD [35]. Taken together, these studies provide conclusive evidence for an inverse relationship between the activation of NA-ergic system and the occurrence of REMS.

Any manipulation of NA-ergic system should also affect the LC target sites, LDT/PPT being one of them, which is relevant for REMS regulation as well. *In vitro* studies have shown that NA inhibits and excites different subpopulations of cholinergic neurons in PPT [36]; however, a direct evidence for physiological regulation of PPT neurons by NA for REMS generation in rats was lacking. To better understand the NA-ergic regulation of PPT for REMS generation, microinjection of agonist/antagonists of NA alone or in combination was done in free moving rats [37]. Intra-PPT microinjection of propranolol (β -antagonist), clonidine (α_2 -agonist), and prazosin (α_1 -antagonist) increased the total time spent in REMS. Propranolol and prazosin affected the mean number of REMS bouts whereas clonidine affected the mean duration of REMS per bout. It was proposed that α_1 - and β -receptors are postsynaptic, present on wake-ON and REM-ON neurons, respectively, whereas α_2 -receptors are present on the presynaptic NA-ergic terminals [37]. Therefore, it was postulated that endogenous NA in PPT acts simultaneously on α_1 - and β -receptors, thereby inhibiting REM-ON neurons and stimulating wake-ON neurons. The activation of α_2 -receptors inhibits the release of NA from the terminals, contributing to the disinhibition of NA-ergic neurons and consequent increase in REMS.

3 Cholinergic Regulation of REMS

A major source of acetylcholine (ACh) in brainstem is the cholinergic neurons located in LDT/PPT [38]. The cholinergic REM-ON neurons in LDT/PPT increase discharge rate prior to the onset of REMS [8, 9] and express c-fos during recovery REMS, following REMSD [39]. Stimulation of LDT/PPT neurons increase the time spent in REMS [40, 41] and lesion studies have also implicated LDT/PPT in REMS regulation [42, 43]. LDT/PPT provides cholinergic inputs to pontine reticular formation (PRF) [13–15]. Stimulation of PPT enhances acetylcholine (ACh) release in the PRF [44], and increased ACh levels in the PRF have been reported to occur during spontaneous REMS [45]. Further, pharmacological enhancement of cholinergic transmission in PRF [46] increased REMS whereas central administration of cholinergic antagonist prior to intra-PRF carbachol injection antagonized the REMS-enhancing effect of carbachol in PRF [47]. Brainstem ACh-esterase activity increased following REMSD, which returned to baseline level after recovery from REMSD [48] whereas inhibiting pontine ACh-esterase activity, thus elevating local ACh levels, increased REMS [49]. Therefore, it is evident that ACh, and hence cholinergic neurons in LDT/PPT, facilitate the generation of REMS.

4 Reciprocal Relationship Between REM-ON and REM-OFF Neurons

As mentioned above, in isolated and independent studies on freely moving cats, extracellular single neuronal activities were recorded from PRF (gigantocellular tegmental field – FTG) and LC; the former increased while the latter decreased

firing during REMS. It was hypothesized that reciprocal interactions between REM-OFF and REM-ON neurons regulate REMS [50, 51]. In the reciprocal scheme, REM-OFF neurons inhibited REM-ON neurons and in addition had a feedback negative loop. Thus, during REMS, continuous activity of REM-ON neurons lead to the gradual incremental excitation of REM-OFF neurons, which in turn then inhibit the activity of REM-ON neurons. Continuous excitation of REM-OFF neurons leads to auto-inhibition through feedback loop, thereby allowing the REM-ON neurons to start firing and occurrence of REMS [50, 51]. Since its proposition, the identity of the pontine REM-ON and REM-OFF neurons has been established. However, as the reciprocal relationship between REM-ON and REM-OFF neurons was based on isolated independent studies, it was imperative to record the REM-ON and REM-OFF neurons simultaneously along with sleep–wake states. Mallick et al recorded the activity of REM-OFF and REM-ON neurons simultaneously during spontaneous sleep–wake states as well as during stimulation induced waking periods and reaffirmed the reciprocal activity profile of REM-ON and REM-OFF neurons [52].

Although the concept put forward by the reciprocal interaction model was validated by subsequent studies, later findings provided additional data, which called for a significant reappraisal of the hypothesis. Egan and North reported that ACh excites the LC NA-ergic neurons, which have been shown to have a REM-OFF profile [10, 11, 53]. Further, LC receives cholinergic afferents [13, 14], and cholinergic receptors are present on the LC neurons [54]. The ACh levels in and around LC increase during REMS [55] while microinjection of cholinergic agonist into LC increases REMS [46, 56]. Those studies clearly demonstrate REMS-promoting effect of ACh in LC. However, ACh is unlikely to be mediating the REMS-enhancing effect by directly acting on the LC neurons because the latter must cease activity to allow the initiation of REMS.

5 GABA-ergic Modulation of LC NA-ergic REM-OFF Neurons for REMS Generation

It is known that in addition to the excitatory cholinergic inputs, LC also has GABA-ergic inhibitory inputs from local GABA-ergic interneurons and GABA-ergic afferents [57, 58]. Therefore, it was suggested that GABA in LC plays a role in ACh-mediated increase in REMS [59]. The role of GABA in LC was supported by the subsequent studies showing that (1) spontaneous REMS is accompanied by an increase in GABA levels in the LC [60], and (2) the GABA-ergic neurons in LC are active during recovery REMS following REMSD [39]. Also, microinjection of GABA-A antagonist into LC decreases REMS [26], whereas iontophoretic application of GABA into rat LC inhibit the NA-ergic neurons [61]. Based on these studies, Mallick et al. hypothesized that ACh in LC from LDT/PPT excites the local GABA-ergic interneurons, which in turn inhibit the LC NA-ergic neurons for the initiation of REMS [62]. To test this hypothesis, agonists/antagonists of GABA and ACh were microinjected either alone or in sequential combinations into LC in chronically prepared free moving rats and sleep–wake states recorded [21].

Infusion of GABA-ergic or cholinergic antagonist into the LC decreased REMS whereas microinjection of either GABA or cholinergic agonist increased REMS. GABA-active agents affected the duration of REMS per bout whereas cholinergic agents affected the number of REMS bouts. The differential effect on the architecture of REMS indicated that whereas ACh in LC regulated the initiation/generation of REMS, GABA is involved in the maintenance (duration per bout) of REMS. Sequential combinations of agonist/antagonists of ACh and GABA done in tandem showed that irrespective of the order of injection, the effect of GABA-active agent always prevailed [21]. Therefore, the site of action of GABA is the final end point (output) in the interaction between the cholinergic afferents from LDT/PPT and the GABA-ergic neurons onto the NA-ergic neurons in the LC.

Besides the local interneurons, LC receives GABA-ergic inputs from PrH [58]. In order to confirm the contribution of GABA-ergic afferents from PrH onto LC neurons, stimulation electrodes in PrH and microinjection guide cannula were implanted in the LC of the same animal. Electrical stimulation of PrH, which should increase GABA levels in LC, increased REMS, which could be blocked by the concurrent microinjection of GABA antagonist into the LC [22]. Further, a recent study showed the presence of neurons with REM-ON profile in brainstem paragigantocellular nucleus (DPGi), which also has GABA-ergic neurons projecting to LC [63]. Therefore, GABA in LC modulates the generation of REMS through a direct action on LC. Acetylcholine from the cholinergic afferents excite at least the local GABA-ergic interneurons to release GABA, which in turn inhibit REM-OFF neurons [21], as was hypothesized earlier [59, 62].

6 GABA-ergic Modulation of PPT Cholinergic REM-ON Neurons for REMS Generation

The GABA-ergic neurons in PPT [38] show enhanced c-fos expression, a marker of cellular activity, during recovery REMS following REMSD [39] as well as carbachol-induced REMS [64]. PPT receives major GABA-ergic afferents from substantia nigra pars reticulata (SNrpr) [65], and the GABA-ergic neurons in SNrpr increase discharge rate during REMS [66, 67]. It was proposed earlier that GABA in PPT may be involved in REMS regulation [21, 62]. Microinjection of GABA-A antagonist into PPT decreased REMS by decreasing the number of REMS bouts [68], whereas injection of GABA-A agonist at the same site increased REMS by increasing the number of REM sleep bouts [69]. Pharmacological stimulation of SNrpr, which should increase GABA levels in PPT, increased the time spent in REMS by increasing the number of REMS bouts, whereas electrical stimulation of SNrpr antagonized the REMS suppressing effect of picrotoxin, GABA-A antagonist, injection into PPT [69]. Our results in rats are supported by previous studies done in cat in which muscimol injections into the PPT of cat increased REMS whereas bicuculline

injections into the same site decreased REMS [70]. Therefore, from these and previous studies it is evident that GABA in PPT promotes REMS [39, 68–70].

As GABA is a known inhibitory neurotransmitter, it cannot excite the REM-ON neurons in PPT to increase REMS. We argued that one possible mechanism through which GABA may exert an excitatory effect on the REM-ON neurons is by inhibiting another inhibitory input (disinhibition) onto the REM-ON neurons. Based on our observation that picrotoxin, GABA-A antagonist, into PPT decreased the total time spent in REMS by decreasing the number of REMS bouts, we postulated that GABA acts presynaptically on the NA-ergic inhibitory inputs onto the REM-ON neurons [68]. In a subsequent study, we showed that clonidine, which acts on presynaptic α_2 auto receptors, in PPT increases REMS by increasing the mean duration of REMS per bout [37]. Interestingly, in the same study, it was observed that coinjection of picrotoxin with clonidine into PPT nullified the previously shown clonidine-induced increase in REMS. A detailed analysis showed that although there was an increase in the duration of REMS per bout as observed after single clonidine injection, there was also a decrease in the number of REMS bouts, as observed previously after a single picrotoxin injection. The coinjection showed the effects attributable to both the drugs, which can happen if the clonidine and picrotoxin act on the same target axon, i.e., presynaptic NA-ergic axonal terminal [37].

It is interesting to note that whereas GABA in LC affected the duration of REM sleep per bout [21, 22, 26, 71], in PPT, GABA affects the number of REMS bouts [37, 68, 69]. Further, unlike LC where ACh excites the GABA-ergic interneurons to release GABA, which then inhibit the LC NA-ergic neurons, GABA-ergic neurons in PPT inhibit the NA release by acting at the presynaptic terminals. Similar GABA-ergic presynaptic mechanism has been shown in PRF where GABA putatively acts on presynaptic level to inhibit the release of ACh [72, 73]. Thus, our findings [37] strongly suggest an inhibitory presynaptic action of GABA on the inhibitory NA-ergic inputs on the REM-ON neurons [37, 68]. The action of GABA on the presynaptic NA-ergic terminals inhibits the release of NA causing withdrawal of inhibition (disinhibition) from the REM-ON neurons resulting in the generation of REMS.

7 Regulation of REMS-Related Neurons by Wake- and Sleep-Inducing Areas for REMS Generation

Despite being mutually exclusive events, REMS and wake states have some apparently common electrophysiological characteristics, viz., desynchronized/active electroencephalogram (EEG) and eye movements. Mallick et al. proposed that (1) separate groups of neurons in the brain stem are responsible for EEG desynchronization observed during wake and REMS, and that (2) the REMS related neurons, i.e., REM-ON and REM-OFF neurons in the brainstem, may be differentially modulated by the brainstem wake-inducing area [52]. To test this hypothesis, surgically prepared chronic cats were instrumented to record sleep–wake states and

single neuronal activity, and in addition, a stimulation electrode was implanted in midbrain reticular formation (MRF) [74]. Earlier studies have consolidated the role of MRF in the induction of EEG desynchronization and waking [75].

On the basis of correlation with the behavioral state, the neurons were classified as REM-ON, REM-OFF, or non-REMS related. Once a single unit stable over the course of at least three sleep–wake periods was encountered, the MRF was stimulated (1 Hz rectangular pulse, 500–600 μ A, 300 μ s) to study the influence of wake-inducing area on each of those neurons. The results showed that MRF stimulation have an excitatory effect on the REM-OFF neurons and inhibitory effect on the REM-ON neurons [74]. A similar combined stimulation and single unit recording study in cats showed that the caudal brainstem, which is known to be a sleep-inducing area [75], excites the REM-ON neurons [76]. On the basis of these findings, it has been postulated that sleep-onset-induced reduction in the activity of MRF neurons [77] produces a gradual reduction in the excitation of the REM-OFF neurons while withdrawing inhibition from the REM-ON neurons. Further, absence of inhibition from wake-inducing area and active excitation from sleep-inducing area contributes to the activation of REM-ON neurons, which leads to the initiation of REMS.

8 A Physiological Model for REMS Regulation

Based on the findings reported by us as well as by others, we put forward the neural connections explaining the generation and regulation of REMS (Fig. 1). The novelties in this model are the brain stem sleep and waking area inputs on REM-ON and REM-OFF neurons and the presynaptic modulation of neurotransmitter release modulating REMS, which we first proposed based on our neuropharmacological in vivo studies [37].

During wake state, the wake-inducing neurons in MRF provides excitatory input to the LC REM-OFF neurons [74], which in turn release NA in PPT to keep the REM-ON neurons inhibited, thus suppressing the occurrence of REMS especially during waking. With the onset of non-REMS, the inputs from extended VLPO exert an inhibitory effect on LC neurons [23, 24], and at the same time, excitatory drive from MRF [77] as well as orexinergic perifornical area [29] onto the LC neurons is reduced. The slowing down of the LC REM-OFF neurons together with the excitation from sleep-inducing area [76] primes the REM-ON neurons to start firing. At the same time, GABA from local PPT interneurons and from SNrpr act on presynaptic NA-ergic axon terminals to further inhibit NA release [37, 68, 69]. The inhibition of NA release from NA-ergic axon terminals causes withdrawal of inhibition from REM-ON neurons; in addition, REM-ON neurons also receive excitatory inputs from the sleep-inducing area in the caudal brainstem, and thus the ACh-ergic REM-ON neurons start firing [76]. The ACh released from the

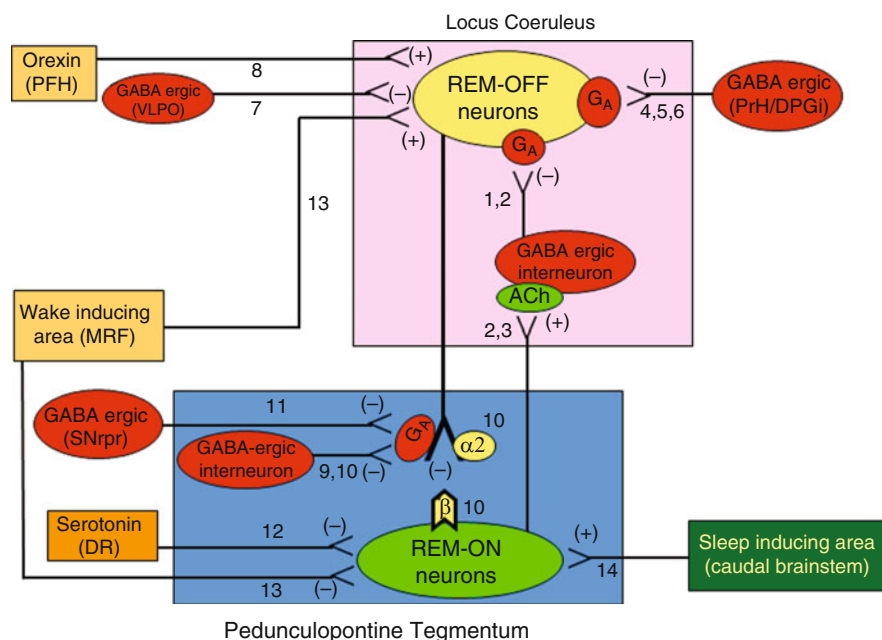


Fig. 1 Schematic representation of the proposed interactions between pontine NA-ergic and cholinergic neurons and their modulation by inputs from other brain regions for REMS generation. *ACh* Cholinergic receptor; *DPGi* Dorsal paragigantocellular reticular nucleus; *G_A* GABA-A receptor; *MRF* Midbrain reticular formation; *PrH* Prepositus hypoglossus; *SNrpr* Substantia nigra pars reticulata; $\alpha 1$ - , $\alpha 2$ - , β -adrenoceptors; (*plus sign*): Excitatory; (*minus sign*): Inhibitory. The proposed connections between different neuronal groups and the interactions between them are based on the following references: (1) [26, 71], (2) [21], (3) [49], (4) [22], (5) [63], (6) [58], (7) [23, 24], (8) [29], (9) [68], (10) [37], (11) [69], (12) [8], (13) [74], (14) [76]

cholinergic REM-ON neurons excites local GABA-ergic interneurons in the LC [21] as well as the GABA-ergic neurons in PrH [22, 78]. As the LC REM-OFF neurons are already primed and slowed down by the inhibitory inputs from sleep area, action of GABA in LC completely suppresses the activity of REM-OFF neurons, allowing the initiation of REMS. Further, the availability of GABA in LC keeps the REM-OFF neurons inhibited, thus maintaining the continuation of REMS.

REMS is a part of the sleep–wake continuum rather than an isolated state because of which it is critical to take into account and integrate the inputs from brainstem sleep–wake regulating areas. As shown in the model (Fig. 1), the physiological data from our laboratory brings out the significance of sleep–wake-inducing areas in the REMS regulation. Further, in addition to establishing a postsynaptic role for GABA in LC for REMS regulation, the model postulates a

presynaptic GABA-ergic modulation of NA in PPT. Although presynaptic modulation of neural activity is ubiquitous in central nervous system, this is the first time that a model based on presynaptic regulation has been envisaged at least for REMS generation.

9 Physiological Validity of the Model

It was proposed that one of the functions of REMS is to maintain neuronal excitability [79]. The excitability of neurons is dependent on the state of membrane potential, which in turn is regulated among other factors, importantly by the activity of Na–K ATPase. Therefore, the activity of Na–K ATPase may be used as a reflection of neuronal and the brain excitability level. In order to investigate the relationship between REMS and neuronal excitability, rats were deprived of REMS and the Na–K ATPase activity estimated from samples prepared from whole brain as well as from specific brain areas. The Na–K ATPase activity increased following REMSD [80–82] and the effect was mediated by associated increase in NA [32–34] by acting on $\alpha 1$ adrenoceptors [83, 84]. As discussed above, NA is released from the LC-REM-OFF neurons, which if not allowed to cease activity by external manipulation, REMS is reduced [18]. Therefore, we argued that activity of LC neurons by modulating the level of NA, which in turn maintains the Na–K ATPase activity in the brain, is likely to be involved in the maintenance of neuronal excitability.

However, sleep is a behavioral phenomenon, which makes it imperative to validate the insights obtained from *in vitro* studies in a freely moving behaving animal preparation. Hence, rats were implanted with electrodes to record sleep–wake behavior and bilateral guide cannula to deliver GABA antagonist bilaterally into LC [71]. We had shown previously that infusion of GABA antagonist, picrotoxin, into LC causes significant loss of REMS for about six postinjection hours [21, 26]. Therefore, repeated picrotoxin injections into rat LC should not allow the LC-REM-OFF neurons to cease activity and cause persistent loss of REMS as was observed during instrumental REMSD in normal rats. Indeed, repeated microinjections of picrotoxin at an interval of six hours for at least 36 h in freely moving, normally behaving rats caused a significant decrease in REMS [71]. Further, the enzymatic analysis of the brains from those rats showed significantly (as compared to control rats) increased Na–K ATPase activity, which was comparable to that observed after instrumental REMSD in normal rats [80, 82]. The findings from this *in vivo* study indeed support our model of neuronal connections at least in the LC and the role of GABA in inhibiting the LC-NA-ergic-REM-OFF neurons for the regulation of REMS.

Acknowledgment Research support to BNM by the CSIR, DBT, DST, and UGC (Networking), India, is acknowledged.

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Involvement of GABAergic Mechanisms in the Laterodorsal and Pedunculopontine Tegmental Nuclei (LDT–PPT) in the Promotion of REM Sleep

Pablo Torterolo and Giancarlo Vanini

Abstract The neurons of the laterodorsal and pedunculopontine tegmental nuclei (LDT–PPT) have a dual function in the control of behavioral states: they promote either wakefulness (W) or REM sleep. During W, these neurons are also related to cognitive and motor functions. In fact, the PPT is the main output station of the basal ganglia circuit and has a major role in the akinesia of Parkinson's disease (PD). Interest in this area has grown tremendously following recent demonstrations that the PPT is a promising target for treating PD symptoms by deep brain stimulation. Further progress in treating PD will be greatly assisted by a clear understanding of the structure and function of the LDT–PPT.

Cholinergic neurons are the principal mediators of activity originating in the LDT–PPT. These neurons project widely to the forebrain and brainstem and increase their firing rate either during W and REM sleep or during REM sleep exclusively. Microinjections of cholinergic agonists into the nucleus pontis oralis, which is a recipient of LDT–PPT cholinergic neuronal projections, prolongs the duration of the REM sleep state. Although GABAergic neurons in the LDT–PPT outnumber cholinergic neurons by two to one, the function of these neurons is mostly unknown. These neurons may have local functions such as controlling the input and the output of cholinergic neurons; however, these cells also project outside the LDT–PPT. Utilizing Fos immunoreactivity as a marker of neuronal activity, it was shown in cats and rats that GABAergic neurons of the LDT–PPT are active during REM sleep. In addition, microinjections of muscimol (GABA_A agonist) within this area generate REM sleep both in cats and rats; on the contrary, GABA_A antagonists induce W. These data suggest that GABAergic

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neurons of the LDT–PPT promote REM sleep. In the present report, we review the role of GABA within the LDT–PPT in REM sleep regulation.

1 Introduction

Since its original discovery, rapid eye movement (REM) sleep has attracted considerable scientific interest because of its paradoxical cooccurrence with activated electroencephalogram (EEG) recordings, which closely resemble a state of wakefulness. Further, most people who are awakened during this state claim that they had just been dreaming [1].

How is REM sleep generated? One important consensus among sleep researchers is that the mesopontine tegmentum contains the necessary and sufficient neuronal networks for REM sleep generation [2]. Within the mesopontine tegmentum, the cholinergic neurons are the defining type of cells of the laterodorsal and pedunculopontine tegmental nuclei (LDT–PPT). GABAergic neurons are also present within the LDT–PPT. In fact, the number of GABAergic neurons is greater than the cholinergic neurons and, further, there is also a considerable GABAergic input to this region. The role of these GABAergic neurons and fibers in the generation and maintenance of REM sleep are the focus of this review.

2 Mesopontine GABAergic Neurons

GABAergic neurotransmission within this region is critically involved in the generation and/or maintenance of REM sleep or wakefulness (W). GABAergic somata and fibers are distributed throughout the mesopontine region [3]. In the nucleus pontis oralis (NPO), microinjections of GABA agonists produce W and block the generation of REM sleep, while GABA antagonists induce REM sleep [4]. Interestingly, one mechanism by which isoflurane induces anesthesia is through a reduction in GABA levels within this site [5]. Further, decreased GABA levels during anesthesia occur in association with reduced cortical excitability, muscular hypotonia, and decreased respiratory rate [5].

Serotonergic neurons of the dorsal raphe nucleus (DRN) regulate sleep and W [6]. Serotonergic neurons in the DRN exhibit a slow tonic rate of discharge during W; their activity decreases during slow wave sleep (SWS) and they cease firing in REM sleep [7]. This suppression of cellular discharge was reversed following the iontophoretic application of GABA_A antagonists into the DRN during REM sleep [8]. In addition, microdialysis experiments have demonstrated

that GABA release in the DRN increases during REM sleep [9]. GABAergic neurons of the DRN are activated during REM sleep in rat and cat, suggesting that local GABAergic neurons are involved in the inhibition of serotonergic neurons [10, 11].

Noradrenergic neurons of the locus coeruleus (LC) also have a “REM sleep-off” pattern of discharge [12]. GABAergic inputs from the dorsal paragigantocellular reticular nucleus of the medulla may be responsible for the inhibition of these neurons during REM sleep [13]. It is accepted that the decrease in noradrenergic and serotonergic activity have a “permissive” effect on neuronal systems that generate REM sleep [14].

GABA_A agonists microinjected into the ventrolateral periaqueductal gray (vlPAG) produce a marked REM sleep hypersomnia in cats and guinea pigs [15, 16]. Although several hypotheses have been proposed, the mechanisms underlying this effect are still unclear.

A cluster of GABAergic neurons is located in the dorsal tegmental nucleus of Gudden (DTN), adjacent to the NPO, LDT, and DRN. Even though the function of the DTN is unknown, it has been demonstrated that GABAergic neurons of this nucleus are active during active W and REM sleep (induced by carbachol), but not during quiet W or SWS [17].

These data demonstrate that the GABAergic neurons and/or GABAergic inputs to the mesopontine regions play a pivotal role for W or REM sleep generation. The question of whether GABAergic neurons in the LDT–PPT also have a role in the control of W or REM sleep is considered below.

3 LDT–PPT: Anatomical Overview

The LDT–PPT, also called CH5 (PPT) and CH6 (LDT), are a loosely defined aggregate of mesopontine cholinergic and noncholinergic neurons [18, 19]. The LDT is located in the central gray, medially to the vlPAG, Barrington nucleus, and LC, and laterally to the DTN and DRN. The PPT neurons lie in close association with the ascending limb of the superior cerebellar peduncle in an area bordered rostrally by the substantia nigra, caudally by the parabrachial nucleus, dorsally by the cuneiform and deep mesencephalic nuclei, and ventrally by the NPO [20–22].

Two divisions were recognized early in the PPT, the pars compacta and pars dissipata, defined by the density of large neurons [23]. These large neurons, which were more concentrated in the pars compacta, were later identified as cholinergic neurons. The area that corresponds with the pars dissipata receives a large projection from the basal ganglia and was termed “midbrain extrapyramidal area (MEA)” by Rye et al. [19]; “noncholinergic area of the PPT” is also used to identify this region [20].

3.1 Neuronal Types

Cholinergic, glutamatergic, and GABAergic neurons are the three main groups of neurons, which make up the LDT–PPT [24]. There are approximately 20,000 cholinergic neurons in the LDT–PPT on each side of the human brain [25]. LDT–PPT cholinergic cells are oval, fusiform, or polygonal in shape, with medium size and 2–3 long (up to 300 μm) primary dendrites [26]. Within the boundaries defined by the cholinergic neurons, a population of smaller noncholinergic cells interdigitate with the cholinergic neurons throughout the LDT and PPT (both in pars compacta and dissipata) [24, 27, 28]. GABAergic neurons within the LDT–PPT are oval or fusiform in shape with a mean large diameter of approximately 13 μm (in comparison with 20 μm of the cholinergic cells) [27]. However, there is an important heterogeneity in the size and shape of the GABAergic neurons [29]. Interestingly, both in the LDT and PPT of the rat, GABAergic neurons outnumber cholinergic neurons by 2:1 [24, 27, 28]. GABAergic neurons are the predominant group in the LDT of the rat [24].

Neurons with glutamate-like immunoreactivity have been visualized in both rats and monkeys [30, 31]. Recently, the presence of glutamatergic neurons within the LDT–PPT was confirmed utilizing *in situ* hybridization for the vesicular glutamate transporter VGlut1; the number of these neurons is comparatively greater than cholinergic neurons [24]. The LDT–PPT also hosts a small number of catecholaminergic neurons [19, 32, 33].

In agreement with the anatomical data, *in vitro* recordings have confirmed the presence of at least three types of neurons within the LDT–PPT, as indicated by the finding of neurons with different voltage sensitive channels [20, 21, 34–36]. However, the neurochemical identification of these neurons remains controversial, and the membrane properties of the GABAergic neurons are still not clear. Glutamic-acid decarboxylase (GAD, the synthetic enzyme for GABA) green fluorescent protein knock-in mice technology, combined with *in vitro* recordings, was utilized to analyze more ventrally and caudally situated GABAergic neurons [37]; these techniques will surely help to characterize the membrane properties and receptor pharmacology of the LDT–PPT GABAergic cells.

3.2 Colocalization of Neurotransmitters Within the LDT–PPT

Nitric oxide, a wide range of peptides, and neurotrophins have been found in cholinergic neurons [20, 21, 38, 39]. Also, glutamate-like immunoreactivity is present in approximately 40% of the cholinergic neurons in the monkey, but only a minor (1–2%) percentage contains the VGlut1 transcript in the rat [24, 40].

In the cat, immunoreactivity against GABA is present in 50% of the cholinergic neurons and in 30% of the cholinergic terminals [41]. However, only $\approx 1\%$ of cholinergic neurons contain a GAD transcript in the rat [24]. In addition, the peptide melanin-concentrating hormone (MCH) has also been found to colocalize with

GABA in LDT neurons of the female rat [42]; this peptide has been recently related to the regulation of sleep [43–45].

3.3 *Input and Outputs of the LDT–PPT*

There are excellent reviews providing detailed descriptions of the inputs and outputs of the LDT–PPT, and the readers are referred to these works [20, 21, 34–36]. In the present report, we will briefly describe the most important LDT–PPT connections (Fig. 1).

Most initial tracing studies have defined the PPT as the pontine region that receives afferents from the basal ganglia, highlighting the importance of these inputs. Projections from the substantia nigra pars reticulata, the internal pallidum, and the subthalamic nucleus are mainly directed to the pars dissipata region of the PPT. Afferents from the cortex, limbic system, cerebellum, sensory pathways, and different areas of the reticular formation have been also described. Due to the role of the LDT–PPT on REM sleep generation, it is important to draw attention to the noradrenergic inputs from the LC and serotonergic inputs from the DRN; both neurotransmitters have inhibitory actions on cholinergic neurons [14].

There are ascending and descending outputs from the LDT–PPT. A large number of neuronal projections are directed toward the thalamus; most of the thalamic projections, which have an important role in EEG activation both during W and REM sleep, are cholinergic. The LDT–PPT also project to different sectors of the basal ganglia and limbic system. Cholinergic projections descending toward the NPO play a critical role in REM sleep generation. There are also LDT–PPT neuronal projections toward the medullary reticular formation and spinal cord. These projections are mainly noncholinergic.

In comparison to neurons in the PPT, LDT neurons have more medial connections and more interactions with the limbic system. For example, while the LDT neurons project toward the dopaminergic ventral tegmental area, the PPT projections are directed mainly toward the dopaminergic substantia nigra pars compacta.

4 Dual Role of the LDT–PPT: Control of REM Sleep and Waking-Related Activities

The LDT–PPT is considered a key part of the ascending reticular activating system; stimulation of this region produces EEG activation and behavioral arousal [46, 47]. This effect is mostly mediated by the projections of the cholinergic neurons toward the thalamus [48]. The PPT has also been involved in specific waking functions, such as locomotion, and is considered an important component of the mesencephalic locomotor region [21]. This functionally defined region is the place where electrical or chemical stimulation generates locomotion in

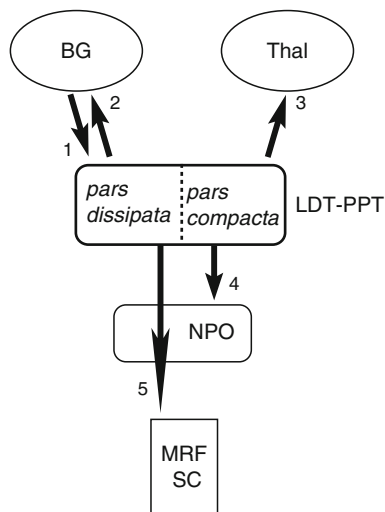


Fig. 1 Scheme of the main connections of the LDT–PPT. (1) Projections from the BG. There is an important GABAergic pathway from the internal pallidum and substantia nigra pars reticulata toward the pars dissipata (“noncholinergic”) region of the LDT–PPT. These projections are involved in motor control and are hyperactive in Parkinson disease. (2) Projections towards the basal ganglia. Several nuclei receive inputs from the LDT–PPT. It is important to highlight the mixed projection (cholinergic and glutamatergic) toward the dopaminergic substantia nigra pars compacta. These projections are important in motor control, motivation, and learning. (3) Projections from the LDT–PPT to the thalamus are mainly cholinergic. These projections are important for the EEG activation that occurs during wakefulness and REM sleep. (4) The cholinergic projections toward the NPO are critical for REM sleep generation. (5) Projections to the MRF and SC arise mainly from the noncholinergic neurons of the pars dissipata. These projections are involved in locomotion and in the control of muscular tone. *BG* basal ganglia; *LDT–PPT* laterodorsal and pedunculopontine tegmental nucleus; *MRF* medullary reticular formation; *NPO* nucleus pontis oralis; *SC* spinal cord; *Thal* thalamus

decerebrate animals. The close anatomical link with the basal ganglia also supports the inference of PPT involvement in motor control. However, a modification of this view is that the basal ganglia have less to do with the control of musculature per se, and much more to do with the selection of appropriate and suppression of inappropriate motor actions, providing a cognitive dimension to the motor activity. In this regard, the PPT has also been implicated in cognitive functions, such as establishing action–outcome associations, reward and learning, activities that have been classically related to the basal ganglia [34, 49].

Presumed LDT–PPT cholinergic neurons either increase their firing rate prior to and during REM sleep (REM-on neurons) or have a larger discharge rate during W and REM sleep than in SWS (Wake/REM-on neurons) [50–52]. Experimental studies have also shown that increases in REM sleep are promoted by electrical or glutamatergic stimulation of the LDT–PPT (with different paradigm of stimulation than the one used to generate W), while neurotoxic lesions of this area decrease

REM sleep [46, 53, 54]. Microdialysis studies have shown that the acetylcholine release in the thalamus increases both in W and REM sleep in comparison to SWS, while only during REM sleep in the NPO [55, 56a]. In the NPO, a single microinjection of a cholinergic agonist, such as carbachol, induces an extraordinary behavioral response in the cat, consisting of the generation of REM sleep with a very short latency (down to 30 s) together with durations that can exceed 2 h [56b]; an example of an REM-carbachol episode was presented in Torterolo et al. [57]. This fact emphasizes the importance of cholinergic projections to the NPO in the generation of REM sleep.

As mentioned above, the role of the LDT–PPT cholinergic neurons in the control of REM sleep and arousal have been well established. On the contrary, the role of the cholinergic neurons in motor and cognitive functions is still not clear. In the following sections, we consider the role of the GABAergic neurons of the LDT–PPT.

5 GABA in the LDT–PPT

5.1 GABAergic Inputs to the LDT–PPT

In addition to local GABAergic neurons already described, GABAergic fibers from sources extrinsic to the LDT–PPT, such as the internal pallidum and the substantia nigra pars reticulata (SNr) have been described [58–60].

Neurons containing MCH are localized within the posterolateral hypothalamus and adjacent areas and project to the LDT–PPT [61–63]. Elias et al. demonstrated in the rat that 25% of the lateral hypothalamic neurons that project to the PPT were immunoreactive for MCH [63]. Interestingly, GABA is also colocalized in MCHergic neurons [42, 64].

Either putative local GABAergic neurons or GABAergic inputs to the LDT–PPT give rise to prominent perisomatic and peridendritic innervation of neurons. In fact, GABAergic terminals comprised about 40% of all terminals within the LDT–PPT [41]. GABAergic terminals synapse with cholinergic and noncholinergic neurons; some of the latter underlie a process of GABA–GABA interaction, an anatomical base for disinhibition [41].

5.2 GABAergic Receptors in the LDT–PPT

GABA_A and GABA_B receptors have been described in the mesopontine area of the rat [65, 66]. However, the relative distribution of GABAergic receptors in cholinergic and noncholinergic neurons, and the subcellular location of these receptors

(pre or postsynaptic, somatic or dendritic) are still unclear. Both receptors have been involved in the control of sleep at the LDT–PPT level (see below). On the contrary, the role of GABA_C receptors in REM sleep control within LDT–PPT seems to be minor [67]. It is unknown if the extrasynaptic GABA receptors regulate sleep in the LDT–PPT [68].

5.3 *Local GABAergic Neurons: Interneurons or Projecting Neurons?*

Utilizing the subunit-B of the cholera toxin as a retrograde tracer, Ford et al. demonstrated in rats that GABAergic neurons of the LDT–PPT project to the posterolateral hypothalamus, where waking and sleep-related hypocretinergic and MCHergic neurons are located [62, 69]. Approximately 20–30% of all the retrograde-labeling neurons were GABAergic, a percentage similar to the retrograde-labeled cholinergic neurons that project in parallel to the same region [27]; in the LDT, approximately 2% of the GABAergic neurons project to this hypothalamic area. GABAergic projections to the subthalamic nucleus have been also identified [70].

Noncholinergic neuronal projections from the LDT–PPT have been identified to different targets such as thalamus, NPO, medullary reticular formation, spinal cord, etc., but what is still unknown is the contribution of the GABAergic neuronal projections to these sites [20, 21, 34–36].

Golgi studies have demonstrated that small neurons similar to GABAergic neurons have axons that terminate locally within the PPT [71]. Terminals containing both acetylcholine transferase (the synthetic enzyme for acetylcholine) and GABA are present within the LDT–PPT; it is believed that these terminals arise from local neurons [41]. It is likely that the LDT–PPT GABAergic neurons modulate sleep both by local and distant projections.

6 Activity of Putative GABAergic Neurons in the LDT–PPT During Sleep and Wakefulness

Utilizing double-labeling immunohistochemical techniques to localize GABA and Fos, Torterolo et al. demonstrated that GABAergic neurons of the LDT and PPT were active during REM sleep induced by microinjections of carbachol into the NPO [72]. In Fig. 2a–h, examples of small GABAergic neurons of the LDT–PPT that are activated during REM sleep are shown. Figure 2j illustrates the fact that the number of active GABAergic neurons is larger during REM sleep in comparison to W.

In agreement with our study in the cat [72], Maloney et al. reported an increase in Fos immunoreactive GABAergic neurons within the LDT–PPT of the rat during the REM sleep rebound following sleep deprivation [11].

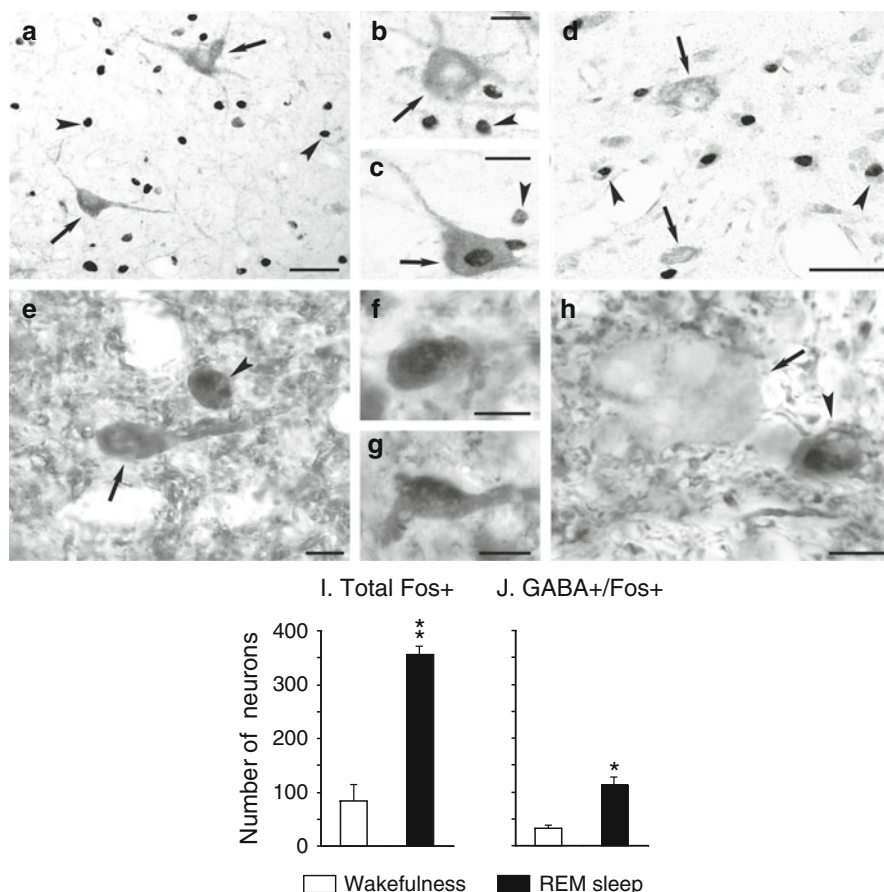


Fig. 2 Photomicrographs illustrating neuronal immunostaining for Fos protein (an index of neuronal activity), acetylcholine transferase (ChAT, to identify cholinergic neurons), and GABA in the LDT-PPT during REM sleep. (a) Double-labeling for ChAT and Fos. Fos immunoreactivity was observed in noncholinergic neurons (indicated by arrowheads). ChAT immunoreactive neurons did not express c-fos (arrows). (b) Photomicrograph exhibiting a cholinergic neuron (arrow) in close proximity to noncholinergic Fos+ neurons (arrowhead). (c) Photomicrograph illustrating a cholinergic neuron that expresses c-fos (arrow) in close proximity to Fos+ neurons (arrowhead). (d) Section immunostained for Fos and counterstained for Pyronin-Y, in which Fos immunoreactivity is observed only in small-sized neurons (arrowheads); larger neurons (arrows, presumptive cholinergic neurons) do not express c-fos. (e) GABA+ Fos+ neuron (arrowhead) that is in close relation to a GABA+Fos negative neuron (arrow). (f and g) Photomicrographs depicting two examples of GABA+Fos+ neurons. (h) GABA+ Fos+ neuron (arrowhead) in the vicinity of a larger, unstained neuron (arrow) surrounded by GABAergic immunoreactive processes. Because of its large size, this neuron is likely to be cholinergic. Calibration bars: a and d, 50 μ m; b and c, 20 μ m; e, f, g, h, 10 μ m. (i and j). Mean numbers of immunoreactive neurons in the LDT-PPT during wakefulness (empty bars) and REM sleep induced by carbachol microinjections into the NPO (filled bars). Compared to wakefulness, there was a statistically significant increase in the mean number of total Fos+ (i) and GABA+ Fos+ neurons (j) during REM sleep. * $P < 0.005$, ** $P < 0.0005$. Modified from Torterolo et al. [72]

In a recent study, LDT–PPT GABAergic neurons were recorded during spontaneous slow waves and somatosensory evoked fast EEG activity in urethane anesthetized rats [29]. The activity of these neurons was heterogeneous; while $\approx 48\%$ of them increased their discharge in relation to EEG activation (similar to cholinergic neurons), $\approx 38\%$ decreased or ceased firing, while the remainder did not change their activity. There are no reports that describe the firing of the LDT–PPT GABAergic neurons during sleep and W.

7 GABA_A Agonists Microinjected into the LDT–PPT Promote REM Sleep

In the cat, activation of GABA_A receptors in the PPT by microinjection of muscimol has been found to decrease the latency, and to increase the percentage of time spent in REM sleep by increasing the frequency and duration of REM sleep episodes. This increase in REM sleep time is at the expense of the time spent in W [73]. Muscimol has also been found to increase the percentage of SWS with pontogeniculooccipital (PGO) waves (a typical sign of REM sleep in the cat), suggesting an increase in REM sleep pressure.

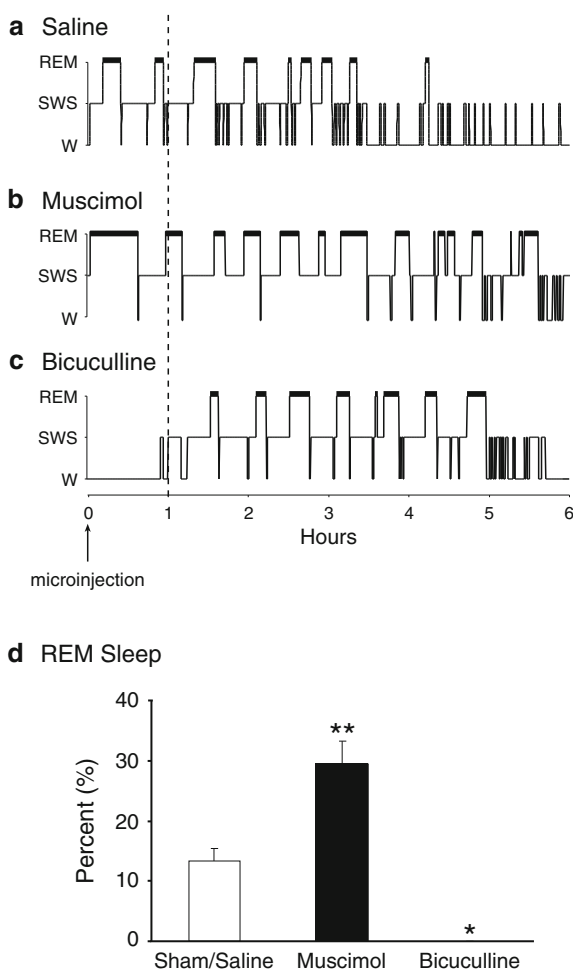
Effects opposite to those following muscimol have been observed following the microinjection of bicuculline, a competitive antagonist of the GABA_A receptor. Bicuculline increased the percentage of time spent in W and suppressed both REM sleep and SWS [73]. Representative hypnograms following control, muscimol and bicuculline microinjections are shown in Fig. 3a–c; the described effects are more pronounced in the first hour following the microinjections (Fig. 3a–d). Another study in cats, however, did not observe this REM sleep-blocking effect of bicuculline [74].

Consistent with the findings of the study in cats, microinjections of GABA_A antagonists (picrotoxin) decreased REM sleep in rats, affecting mainly the mean number of REM sleep bouts [75, 76]. Furthermore, the GABA_A agonist muscimol increased REM sleep without affecting any other behavioral state [77]. This increase in REM sleep was found to be due to increases in the mean number of REM sleep episodes. Surprisingly, microinjections of another GABA_A agonist (isoguvacine) did not produce any effect on sleep [67].

8 Effects on Sleep of GABA_B and GABA_C Agents Microinjected into the LDT–PPT

In rats, when GABA_B receptors were activated by a local microinjection of a GABA_B receptor selective agonist (baclofen), REM sleep was suppressed in a dose-dependent manner [67]. This effect is mediated by the inhibition of the

Fig. 3 Effects on sleep produced by the microinjection of muscimol and bicuculline into the PPT of the cat. **(a)** Hypnograms during a control experiment (saline microinjection). **(b)** Muscimol (20 mM) produced an increase in the number of REM sleep episodes as well as an increase in the duration of the first REM sleep episode. **(c)** Bicuculline (15 mM) suppressed REM sleep during the first recording hour. The *vertical dashed line* delimits the first recording hour. The *arrow* indicates the end of the microinjection procedure. **(d)** Bar chart that highlights the percentage of REM sleep during the first recording hour following sham/saline, muscimol and bicuculline microinjections. * $P < 0.01$; ** $P < 0.001$. Modified from Torterolo et al. [73]



intracellular cAMP-dependent protein kinase A signaling pathway [78]. In contrast, when GABA_C receptors were activated by microinjecting the receptor-selective agonist (*cis*-4-aminocrotonic acid), the total percentages of REM sleep did not change compared with the control values [67].

9 GABAergic Inputs to the LDT-PPT Regulate REM Sleep

We mentioned before that there is an important GABAergic input from the SNr. Recently, it was shown that chemical stimulation of this area (that supposedly promotes the release of GABA into the LDT-PPT) enhanced REM sleep by

increasing the mean number of REM sleep bouts; this result was similar and comparable to the effect of the GABA_A agonist muscimol microinjections into the PPT [77]. Electrical stimulation of the SNr did not produce any significant change in the sleep–wake states, although it was sufficient to counter the REM sleep-decreasing effect of microinjections of GABA_A antagonists into the PPT [77].

10 Role of GABA in the LDT–PPT in REM Sleep Generation: Working Hypotheses

We mentioned above that the LDT–PPT GABAergic neurons differ in their morphological and physiological properties. GABAergic neurons project in parallel to cholinergic neurons to different targets, and/or, have local connections for regulating cholinergic and/or glutamatergic outputs. In addition, there are a large number of GABAergic inputs to the LDT–PPT. Thus, the whole scenario of the GABAergic function is not simple. A summary of the main GABAergic connections implicated in the modulation of REM sleep by the LDT–PPT is presented in Fig. 4.

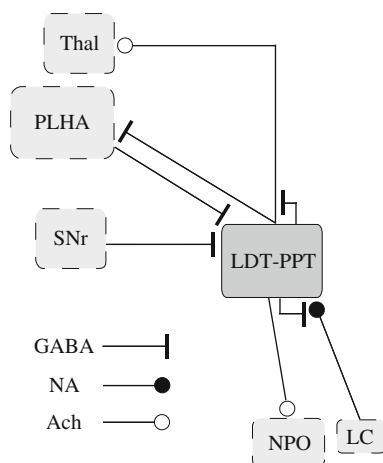


Fig. 4 GABAergic afferents, efferents, and interneurons within the LDT–PPT involved in the modulation of REM sleep. Putative GABAergic neurons control the inputs and the outputs of the LDT–PPT. Pal and Mallick’s hypothesis of the GABAergic control of noradrenergic release at the PPT is depicted. GABAergic inputs from the SNr that are known to regulate REM sleep are also illustrated. In addition, cholinergic outputs to the thalamus and the NPO, critical for REM sleep generation are also presented. *LC* locus coeruleus; *LDT–PPT* laterodorsal and pedunculo-pontine tegmental nucleus; *NPO* nucleus pontis oralis; *PLHA* posterior-lateral hypothalamic area; *SNr* substantia nigra pars reticulata; *Thal* thalamus

Although the number of the LDT–PPT GABAergic neurons that express Fos increase during REM sleep, the pattern of activity of these cells during this state is still unknown. Neurons with brief action potentials and high frequency discharge, which are presumably noncholinergic, are active during REM sleep [50, 79, 80]. These neurons abruptly cease firing during PGO waves. It has been proposed that these REM-on, PGO-off neurons are GABAergic, and they are thought to disinhibit adjacent PGO-on neurons [80]. Presumed GABAergic neurons of the SNr that project to the PPT have been also recorded. Some of these neurons exhibited an increased firing rate, preceding by 70–200 ms the thalamic PGO waves [81]. The authors suggest that an enhancement in SNr-cells' discharges may lead to hyperpolarization of PPT neurons, with the consequence of spike bursts in one class of PGO-related PPT neurons. Therefore, electrophysiological data suggest that local GABAergic neurons and GABAergic inputs may be essential for one important component of REM sleep: the PGO waves.

It is also possible that the LDT–PPT GABAergic neurons suppress neighboring REM sleep-off monoaminergic neurons during REM sleep. This function may be stronger in the cat, in which there is substantial intermingling of cholinergic and noradrenergic neurons [32].

Increases in REM sleep time occur following the microinjection of GABA_A agonists into the PPT or by the stimulation of the GABAergic inputs from the SNr, and decreases are produced by microinjections of GABA_A antagonists (see above). If we consider the LDT–PPT solely as a REM generation area, this fact could be viewed as being contrary to expectations. However, we have already noted that the LDT–PPT is a heterogeneous area, in which a waking-related and an REM sleep-related domain, with contrasting and mutually exclusive functions, coexist. In this regard, disinhibition of the PPT or adjacent areas with GABA_A antagonists increases locomotor activity, an action that is compatible with an increase in W, while inhibition by muscimol or GABA decreases such activity [21, 82]. It is thus likely that GABAergic agonists produce a stronger suppression of the waking-related components of the PPT than of the REM sleep-related components; hence, the REM sleep-related mechanisms are released to a greater extent. The opposite effects arise as a result of disinhibition by bicuculline. Pal and Mallick suggested a complementary explanation of the REM enhancing effect of GABA_A agonists [75–77]. These authors hypothesized that GABA_A agonists microinjected into the PPT may promote REM sleep by inhibiting through presynaptic receptors the waking-promoting noradrenergic release within the PPT; these effects would release the REM-sleep component (probably cholinergic) and promote REM sleep. On the other hand, GABA_A antagonist would facilitate the noradrenergic release promoting W.

Interestingly, GABA_B produces effects that are opposite to those of GABA_A agents, probably because of the different cellular location of the receptors. However, it is important to note that small differences in the microinjection site as well as in the dosage of the drugs may produce different effects. For example, glutamate stimulation of the PPT can promote both W and REM sleep depending on the dose

employed [47]. Therefore, it is clear that further research is needed to understand the role of GABA within the LDT–PPT.

11 GABA in the LDT–PPT: Pathophysiology

A loss of cholinergic neurons in the PPT, as well as a correlation between the amount of cholinergic loss and motor symptoms, was demonstrated in Parkinson's disease (PD) and Parkinsonian syndromes [36, 83]. Furthermore, severe akinetic symptoms have been reported after small ischemic lesions of the PPT in humans, and in experimental lesions or inactivation of the PPT in monkeys [36, 83]. In addition, in both Parkinsonian monkey and humans, there is an abnormally high GABAergic neuron activity in the internal pallidum, which inactivates the neurons of the PPT through direct projections [83]. Thus, the GABA-mediated decrease of the activity of the PPT may underlie the pathophysiology of PD; in fact, the beneficial effect of the lesion of the subthalamic or internal pallidum as well as of the high-frequency stimulation of these areas (that is believed to have an inhibitory effect on putative neurons) is achieved by reducing the descending GABAergic influence from the internal pallidum to the PPT [84]. Indeed, micro-injection of bicuculline into the PPT of nonhuman primates with experimental Parkinsonism resulted in significant improvement of akinesia [85]. Appreciation of the importance of the PPT has recently increased because this area is now a target for deep brain stimulation; this procedure has been found to alleviate the akinesia, gait, and postural instability in PD patients and in animal models of PD [85–87].

Most PD patients have sleep disorders that include not only difficulty falling or remaining asleep, but also excessive daytime sleepiness and abnormal events during sleep such as REM sleep disorder [88]. It is likely that the cholinergic neuronal loss and the increase in GABAergic input to the PPT are related to these disorders.

12 Conclusions

Local GABAergic neurons as well as GABAergic inputs are an important component of the LDT–PPT neuronal network. Several lines of experimental evidence support the hypothesis that GABA in the LDT–PPT has a critical role in the generation of REM sleep. In addition, GABA within this area may contribute to the pathophysiology of PD, and possibly contribute to the sleep disorders characteristic of this pathology.

Acknowledgments We thank Dr. Patricia Lagos and Dr. Holly N. Brevig for their critical comments on the manuscript.

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GABAergic Mechanisms in the Ventral Oral Pontine Tegmentum: The REM Sleep-Induction Site – in the Modulation of Sleep–Wake States

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Abstract The ventral part of the oral pontine reticular nucleus (vRPO) is a nodal link in the neuronal network responsible for the generation and maintenance of rapid eye movement (REM) sleep and is reciprocally connected with structures involved in the control of wakefulness and non-REM (NREM) sleep. Shifting from one sleep state to another depends on the balance between excitation and inhibition in the reciprocal connections between these structures. Gamma-aminobutyric acid (GABA) occupies an outstanding place in these processes. In this review, we report the important role of GABA in vRPO function, specifying and discussing its location, origin, and role in this nucleus. Results from our laboratory revealed a few small GABA-immunoreactive cell bodies in vRPO; but we estimated that 30% of all vRPO synaptic terminals are actually immunoreactive to GABA. These inhibitory GABAergic terminals directly target somata and the different segments of the vRPO dendritic tree neurons. GABAergic terminals innervate serotonergic, GABAergic, and no-immunoreactive dendrites and terminals in the vRPO. Functionally, GABA elicits long-lasting hyperpolarizations of vRPO neurons in intracellular recording experiments in vitro. Small-volume microinjections of the GABA_A receptor agonist muscimol in vRPO produce a statistically significant decrease of REM sleep proportions in freely moving cats. GABAergic axon terminals mainly originate in neurons located in diencephalic structures related with NREM sleep (such as the reticular thalamic nucleus) and wakefulness (such as the lateralposterior hypothalamus). These terminals would inhibit the vRPO REM “on” neurons either directly through postsynaptic mechanisms and/or indirectly through presynaptic inhibition of the excitatory terminals that form synapses with these neurons. Non-GABAergic and nonserotonergic terminals making asymmetric synapses (putatively excitatory) would indirectly inhibit REM “on” neurons

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through excitation of both serotonergic and GABAergic neurons, and terminals in the vRPO. Inhibition of GABAergic transmission in the vRPO contributes to the generation and maintenance of REM sleep.

1 Introduction

The ventral part of the oral pontine reticular nucleus (vRPO) is the only region of the brainstem in which small volume microinjections of low doses of the cholinergic agonist carbachol produce all the bioelectrical and behavioral signs of rapid eye movement (REM) sleep with a short latency in freely moving cats (Fig. 1) [1–4]. In addition, there are other neurotransmitters that trigger or inhibit REM sleep generation through the activation or inhibition of neurons in both the cat vRPO and its equivalent in the rat [5–13]. Lesions located in the cat vRPO produce a specific and significant decrease in REM sleep [14, 15]. Early lesion studies attributed the organization of REM sleep to structures located in the oral pontine reticular nucleus (RPO) [16]; moreover, clinical studies have reported alterations in REM sleep in

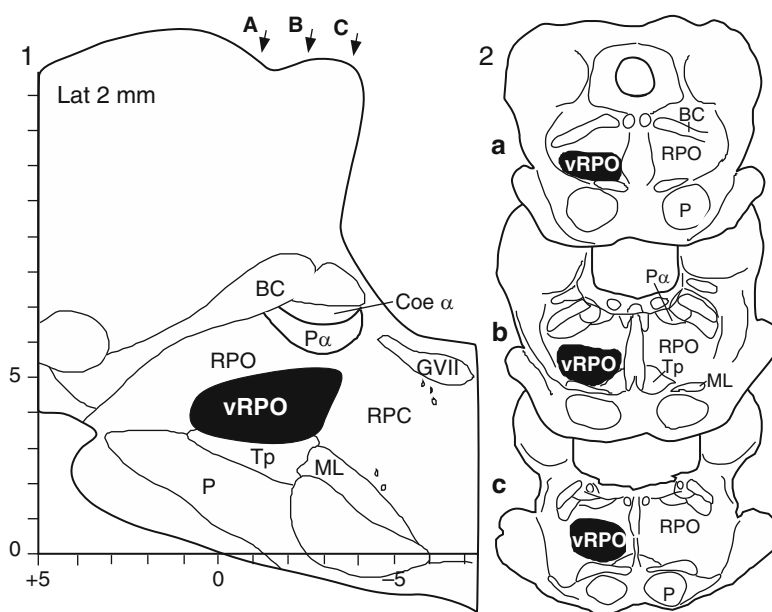


Fig. 1 Schematic representation of the situation and limits of the nodal structure of the neural network responsible for the organization of REM sleep in the ventral part of the oral pontine reticular nucleus (vRPO). (1) A parasagittal brainstem section shows the location and extent of vRPO, (2) in three coronal brainstem sections made to levels A–C in the parasagittal section (see part 1). BC brachium conjunctivum; Coe α locus coeruleus α ; GVII genu of the facial nerve; ML medial lemniscus; P pyramidal tract; P α perilocus coeruleus α ; RPC caudal pontine reticular nucleus; RPO oral pontine reticular nucleus; Tp tegmentopontine nucleus

patients with lesions in the ventral pontine tegmentum [17, 18]. Consequently, we consider the vRPO a nodal link in the neuronal network responsible for the generation and maintenance of REM sleep.

The vRPO can efficiently and harmoniously orchestrate and coordinate the wide neuronal network responsible for inducing and maintaining REM sleep because it is connected with other brainstem structures, each of which is basically responsible for one of the different bioelectrical signs that define REM sleep [1, 2, 19]. On the other hand, the equilibrium between the excitatory and inhibitory actions of different neurotransmitters from structures that project to the vRPO may be determinant for vRPO-REM sleep generation (Fig. 2) [1, 2].

The vRPO is also reciprocally connected with prosencephalic structures involved in the control of the other states of the sleep–wakefulness cycle, namely, wakefulness and non-REM (NREM) sleep (Fig. 2) [1, 19]. Shifting from one state to another probably depends on the balance between excitation and inhibition in the reciprocal connections between these structures, all of them within a circadian cycle controlled by a pacemaker: the suprachiasmatic nucleus. It has been demonstrated that the suprachiasmatic nucleus has an important role in the active promotion of REM sleep at specific times of the day [20].

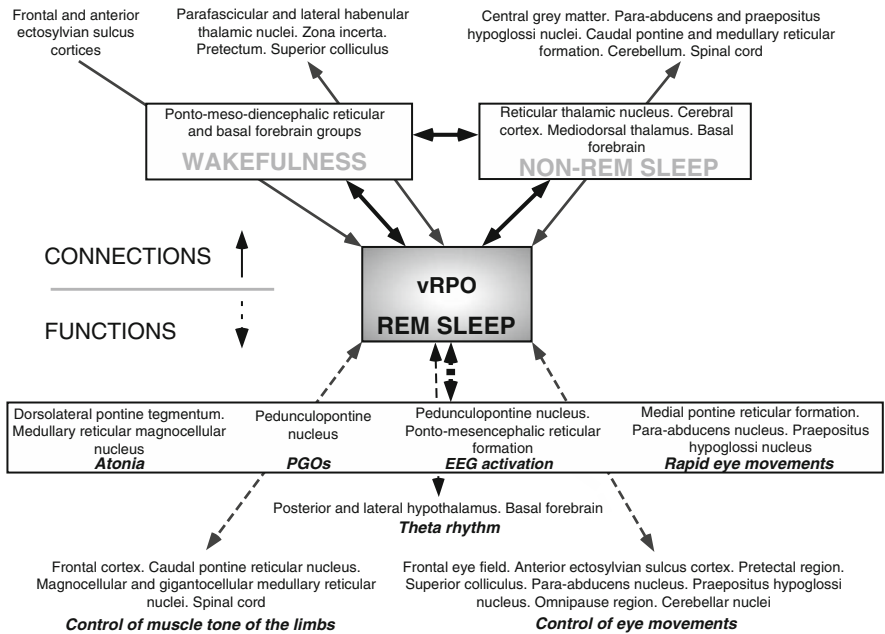


Fig. 2 Schematic representation of the neural network responsible for the organization of REM sleep. The vRPO is closely connected with the structures responsible for the different signs of REM sleep: atonia, PGOs, activation of the EEG, rapid eye movement, and theta rhythm. This structure is also closely connected to the structures responsible for other phases of the sleep–wakefulness cycle as well as numerous other central nervous system structures, all of which modulate the activity of its neurons. *EEG* electroencephalogram; *PGOs* pontogeniculooccipital waves [29]

Gamma-aminobutyric acid (GABA) is the most widely distributed inhibitory neurotransmitter in the central nervous system [21–23]. It seems to occupy a key role among the neurotransmitters in decreasing vRPO neuron activity, thereby preventing the induction of REM sleep during wakefulness or NREM sleep states.

Numerous experiments by our group and others support the important role of GABA in the behavior of neurons localized in the REM sleep induction site. Here, we shall summarize the most interesting findings regarding the location, origin, and role played by the inhibitory neurotransmitter GABA in this nucleus.

We will divide the exposition into the following sections:

Location of GABA in the vRPO

Origin for GABA in the vRPO

Effect of GABA on vRPO

Final considerations

2 Location of GABA in the vRPO

Previous light [24, 25] and electron microscope studies [26–28] have described GABA-immunoreactive (GABA-IR) neurons and/or terminals in the vRPO. By using a combination of the physical dissector, electron microscopy, and postembedding immunogold techniques, De la Roza and Reinoso-Suárez [23] have recently demonstrated that 30% of all synaptic terminals in the cat vRPO are immunoreactive for GABA. These terminals form symmetric (probably inhibitory) synapses on vRPO somata and dendritic trees, including distal regions (Fig. 3). The synaptic and morphological characteristics of GABA-IR terminals in the cat vRPO are consistent

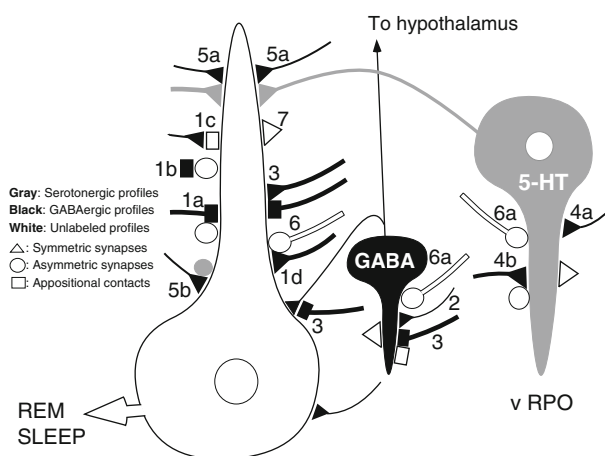


Fig. 3 Schematic representation of the organization of the GABAergic profiles on the neurons and terminals of the vRPO. For details, see text. Done with the data provided by [23, 25, 30]

with the features of the type III and IV terminals previously described by De la Roza and Reinoso-Suárez in cats [29].

Although most of the GABA-IR synaptic terminals were observed on dendrites, 7% of them innervated the somatic compartment of vRPO neurons (Fig. 3). In addition, the mean percentages of GABAergic synapses decreased from large-diameter to small-diameter dendrites; this result agrees with the distribution of all the symmetric synapses on vRPO dendrites [29]. Our data support the hypothesis that the activity of vRPO neurons is significantly controlled by inhibitory GABAergic terminals that directly target neuronal somata and dendritic trees. In addition, the GABAergic axon terminals in the cat vRPO were often located next to unlabeled asymmetric (probably excitatory) synapses (Fig. 3, label 1a), suggesting that the activation of the vRPO dendrites by the terminals which form asymmetric synapses may be decreased by the activation of nearby GABAergic-inhibitory synapses [23].

Light microscopic immunocytochemistry revealed a few GABA-IR cells scattered throughout the vRPO, and these cells were usually small and oval to fusiform in shape [23]. These vRPO GABAergic neurons could represent local circuit neurons that would contribute, although only to a limited degree, to the GABAergic innervation of the vRPO (Fig. 3). However, they could also correspond to the GABAergic pontine neurons that project to the rat posterolateral hypothalamus [25] (Fig. 3). Supporting the latter possibility, the De la Roza team (unpublished results) has observed presynaptic dendrites in the vRPO neurons that project to the posterolateral hypothalamus.

De la Roza and Reinoso-Suárez have recently found that vRPO GABA-IR neurons are innervated by symmetrical synaptic GABA-IR terminals [30]. Only 7.5% of the synapses formed by these GABA-IR terminals were located on GABA-IR dendrites (Fig. 3, label 2); in addition, GABA-IR terminals were sometimes found in close apposition to one another (Fig. 3, label 3). Although these results indicate the presence of disinhibitory GABAergic mechanisms, the number of such contacts actually visualized was very low. Furthermore, some GABA-IR terminals made symmetric synapses (3.5%) or were apposed to unlabeled synaptic terminals that sometimes formed asymmetric synapses on nonlabeled neurons (Fig. 3, labels 1b–1d) [30].

Using double immunolabeling staining for 5-HT and GABA, De la Roza and Reinoso-Suárez [30] have shown that GABA-IR terminals form symmetric synaptic contacts with 5-HT-IR dendrites (Fig. 3, labels 4a and 4b) that, in some cases, were apposed to asymmetric unlabeled terminals (Fig. 3, label 4b). About 12% of the 5-HT-IR neuron membrane was covered by GABA-IR terminals. In addition, 5-HT-IR and GABA-IR synaptic terminals were seen converging on the same dendrites, but, although the terminals were closely apposed to each other, no synaptic contact was observed between them (Fig. 3, label 5) [30]. These 5-HT-IR terminals made symmetric or asymmetric synapses, while GABA-IR terminals only formed symmetric synapses (Fig. 3, labels 5a and 5b). Unlabeled afferents made asymmetric (Fig. 3, labels 6 and 6a) as well as symmetric synapses (Fig. 3, label 7) on unlabeled, GABA-IR and 5-HT-IR neurons.

3 Origin for GABA in the vRPO

Rodrigo-Angulo et al. [31] did an anatomical study mapping the local origin of the GABAergic basal forebrain and diencephalon projections to the vRPO. They used a double-labeling technique combining vRPO injections of the neuronal tracer, cholera-toxin (CTB), with GAD-immunohistochemistry in adult cats. Double-labeled CTB/GAD-positive neurons were detected in diencephalic structures. In the thalamus, the reticular thalamic nucleus displayed a considerable number of CTB/GAD-positive neurons across its ipsilateral anteroposterior extent. At rostral levels, the neurons occupied the core of the reticular thalamic nucleus, while at caudal levels, they appeared in its infrageniculate sector and always intermingled with GAD-positive neurons (Fig. 4, parts 1–4). Obviously, as no single-labeled CTB-positive neuron was identified in this structure, the entire projection from the reticular thalamic nucleus to the vRPO was GABAergic. Other thalamic structures showed only a few CTB-positive neurons and no double-labeled neurons.

The ipsilateral zona incerta contained a large number of double-labeled CTB/GAD neurons intermingled with numerous single-labeled neurons, either CTB or GAD-IR, as well as profuse passing fibers [31]. In this case, a few double-labeled neurons were detected in the contralateral zona incerta (Fig. 4, parts 3–5). Only 15.3% of the zona incerta neurons projecting to the vRPO were GABAergic [31].

In the hypothalamus, CTB-positive neurons were very numerous in both the ipsi- and contralateral sides, while double-labeled CTB/GAD-positive neurons were remarkably less abundant, basically being observed in the dorsal hypothalamic area, most abundantly in its caudal sectors (Fig. 4, parts 3–4). They constituted 14.7% of the projection to the vRPO. One sixth of the retrogradely labeled neurons distributed in the lateral hypothalamic area were also CTB/GAD-positive, but only a few double-labeled neurons could be observed in the paraventricular hypothalamic nucleus, H1 Forel field, or perifornical area [31]. A large number of CTB/GAD-positive neurons filled the dorsocaudal hypothalamic nucleus in both the ipsi- and contralateral sides; however, not one single-labeled CTB-positive neuron could be detected in the nucleus (Fig. 4, parts 4–5) [31].

De la Roza et al. [28] examined the morphology and synaptic organization of posterolateral hypothalamic terminals in the vRPO using posterolateral hypothalamic microinjections of biotinylated dextran amine as well as of wheat germ agglutinin conjugated to horseradish peroxidase. A postembedding immunogold technique was used to determine which anterogradely labeled terminals were GABA-immunopositive. The electron microscope analyses revealed a variety of ultrastructural features in the anterogradely labeled vRPO terminals. Double-labeled hypothalamic terminals were always observed on vRPO dendrites and the data showed that about 26% of the posterolateral hypothalamic-labeled vRPO terminals were GABA immunoreactive and formed symmetric synapses on dendrites [28]. These results are consistent with those presented above of the presence of GABAergic neurons in the cat hypothalamus and the fact that GABA is one of the transmitters used by the hypothalamic cells projecting to the vRPO. Moreover,

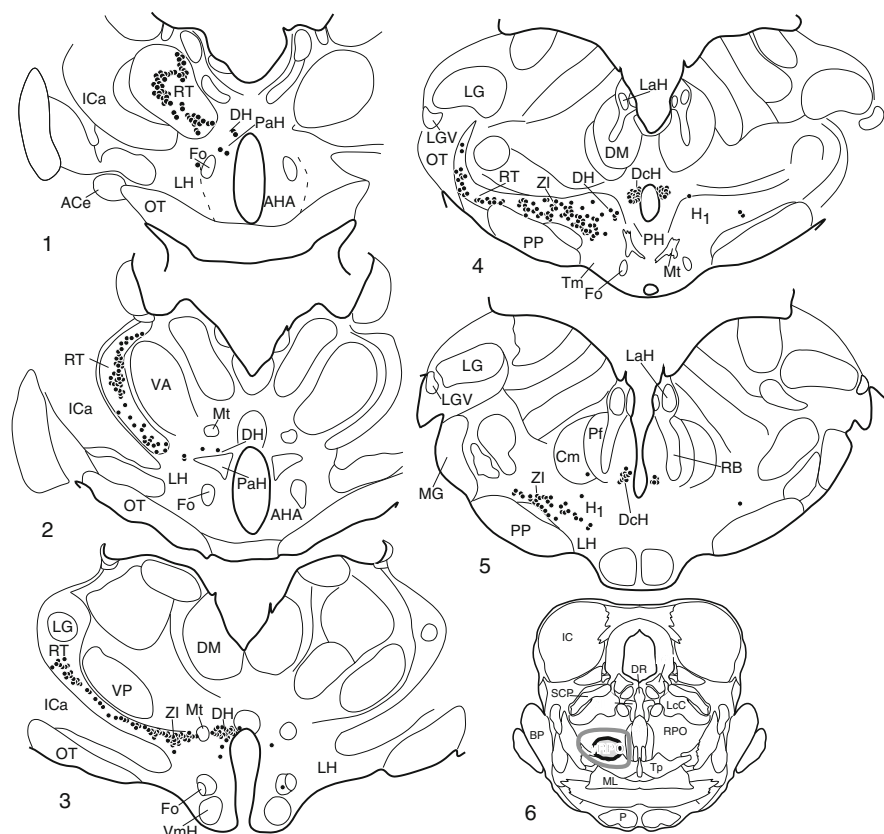


Fig. 4 Distribution of GABAergic neurons projecting to vRPO from diencephalic structures. Drawings schematize coronal sections across the diencephalon from rostral (1) to caudal (5) showing the distribution of CTB/GAD diencephalic double-labeled neurons after the CTB injection in the ventral part of the oral pontine reticular nucleus (vRPO) represented in (6) with a thicker trace and a shaded core. Each dot represents one neuron. *ACe* central amygdaloid nucleus; *AHA* anterior hypothalamic area; *BP* brachium pontis; *Cm* centromedian thalamic nucleus; *CS* central superior raphe nucleus; *DcH* dorsocaudal hypothalamic nucleus; *DH* dorsal hypothalamic area; *DM* dorsomedial thalamic nucleus; *DmH* dorsomedial hypothalamic nucleus; *DR* dorsal raphe nucleus; *Fo* fornix; *H₁* H₁ Forel field; *IC* inferior colliculus; *ICa* internal capsule; *LaH* lateral habenular nucleus; *LcC* locus coeruleus complex; *LdT* laterodorsal tegmental nucleus; *LG* lateral geniculate nucleus; *LGV* ventral lateral geniculate nucleus; *LH* lateral hypothalamic area; *MG* medial geniculate nucleus; *ML* medial lemniscus; *MLF* medial longitudinal bundle; *MN* mammillary nucleus; *Mt* mammillothalamic tract; *OT* optic tract; *P* pyramidal tract; *PaH* paraventricular hypothalamic nucleus; *Pf* parafascicular thalamic nucleus; *PH* posterior hypothalamic area; *PP* pes pedunculi; *RB* retroflex bundle; *RPO* oral pontine reticular nucleus; *RT* reticular thalamic nucleus; *SCP* superior cerebellar peduncle; *Tm* tuberomammillary nucleus; *Tp* tegmentopontine nucleus; *VA* ventral anterior thalamic nucleus; *VmH* ventromedial hypothalamic nucleus; *VP* ventral posterior thalamic nucleus; *ZI* zona incerta. Done with the data provided by [31]

the authors showed that GABA immunonegative hypothalamic terminals forming asymmetric synapses on dendrites were adjacent to GABAergic terminals located on the same dendrite (Fig. 3, labels 1a, 1b, and 1d). Consequently, as suggested in the previous section, the excitatory activation of the vRPO dendrites by the hypothalamic fibers forming asymmetric synapses on the dendrites could be decreased by the activation of the nearby GABAergic inhibitory synapse [23].

Using the CTB/GAD double-labeling technique, Rodrigo-Angulo and Heredero (personal communication) have observed the presence of a few brainstem GABAergic neurons projecting to the vRPO that are scattered throughout the midbrain, pontine (including the contralateral vRPO), and medulla oblongata reticular formations.

4 Effect of GABA on vRPO

This GABA found in the vRPO neurons and synaptic terminals, which originates either in vRPO GABAergic neurons or in GABAergic neurons located at a distance: how does it modulate the vRPO neurons that generate and maintain REM sleep? Our studies *in vitro* using intracellular recordings in slices and studies *in vivo* using the whole animal addressed this question.

4.1 Intracellular Unit Recordings In Vitro

A group of studies in our laboratory described the morphological and electrophysiological characteristics of the vRPO neurons and the action of different neurotransmitters, including GABA, on vRPO neurons *in vitro* [32, 33]; GABA studies were done using intracellular recordings on brainstem 300–400- μm -thick coronal slices of 8–12-day-old Wistar rats [33]. The application of GABA on the vRPO neurons ($n = 14$) in the recording chamber elicited long-lasting hyperpolarizations, associated with a decrease in the R_{in} (5–20%) (Fig. 5a–b). The hyperpolarizations had an amplitude of 10–25 mV. To investigate the possibility that the hyperpolarizations were mediated through the activation of K^+ conductances, the reversal potential of the GABA-evoked hyperpolarizations was determined. The magnitude of the hyperpolarization decreased as the cell was hyperpolarized, reaching the reversal potential at -90 mV. Thus, results suggested that K^+ currents were involved in generating the hyperpolarization, probably through the activation of GABA_B receptors. In order to ascertain if GABA effects were also mediated by activation of Cl^- conductances, intracellular recordings were performed with KCl filled micropipettes: the amplitude of the GABA-evoked hyperpolarization gradually reduced over time after cell impalement, suggesting that the Cl^- leaking from the pipette modified the Cl^- equilibrium potential. The reported neurotransmitter

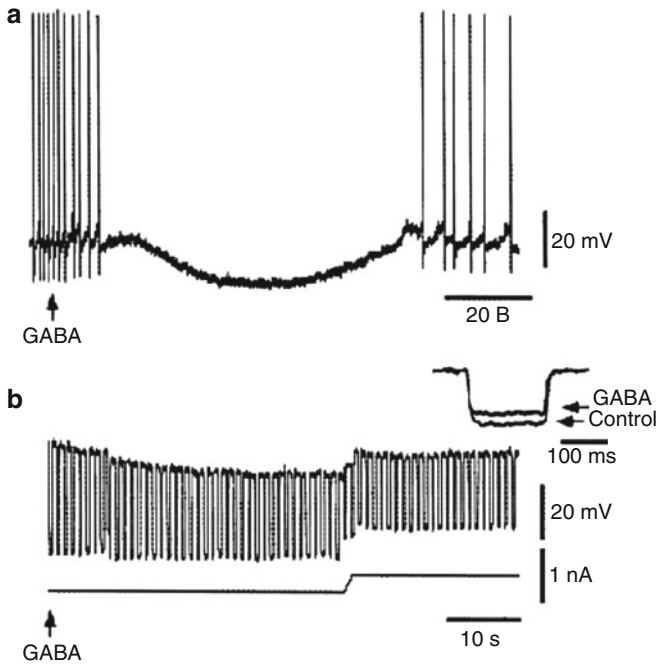


Fig. 5 GABA-evoked hyperpolarization in vRPO neuron during drug application. (a) Hyperpolarizing response was evoked by GABA (4 μ M). (b) In the same neuron, brief hyperpolarizing pulses injected before and during the GABA-evoked hyperpolarization (0.2 nA, 500 ms). Lower traces display the concurrent monitor of the sustained current injection. Insets in **b** show the voltage-deflections evoked by current injection at the same V_m . V_m : -68 mV in **a** and -72 mV in **b** [33]

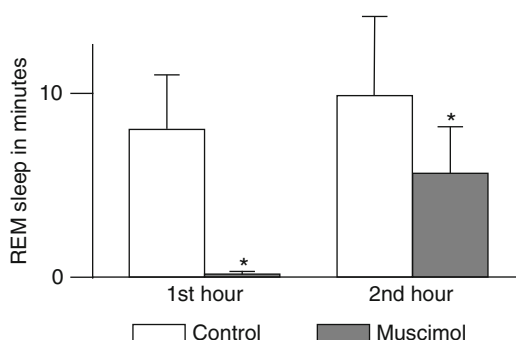
actions were insensitive to TTX (0.5 μ M), demonstrating that GABA acted at postsynaptic membrane sites [33].

Consequently, we concluded that GABA inhibits vRPO cells through the activation of GABA_A and GABA_B receptors [33].

4.2 Studies In Vivo

Drugs that enhance the actions of GABA, such as benzodiazepines and barbiturates, generally increase NREM sleep and decrease REM sleep when administered systemically to humans or animals. However, when injected intracranially to experimental animals, the effects of GABAergic drugs on the sleep–wakefulness cycle vary significantly depending on which brain region is injected. GABA microinjections have been performed in the rat and the cat RPO in vivo [10, 12, 34]. In our laboratory, Manquillo [10] demonstrated that the microinjection of a small

Fig. 6 Statistically significant decrease of REM sleep in the first and second hour of the record after the microinjection of the GABA_A receptor agonist muscimol (20 nl, 50–250 nM) in vRPO in a group of three freely moving cats with chronically implanted electrodes. (*) $p < 0.05$. Done with the data provided by [10]



volume (20 nl) of the GABA_A receptor agonist muscimol in vRPO in chronic cats produced a significant decrease of REM sleep (Fig. 6) with increased wakefulness in the first 2 h after the injection. Injection in the cat RPO by Xi et al. [34] of GABA or muscimol also produced a decrease of REM sleep and prolonged episodes of wakefulness, while injection of the GABA_A receptor antagonist bicuculline produced an increase in REM sleep. Nevertheless, microinjection, by the same authors, of glycine, another major inhibitory neurotransmitter, and its antagonist strychnine into the same area had no effect on the states of REM sleep and wakefulness [34]. Also dialytic administration of bicuculline in the RPO enhanced REM sleep in the cat [35]. Similar phenomena – muscimol injections decreasing REM sleep and bicuculline increasing REM sleep occur after injections in the site equivalent to vRPO in the rat brainstem; moreover, bicuculline injections in the oral pontine reticular formation were more effective for enhancing REM sleep than those made in the caudal pontine reticular formation [12]. The above results indicate that GABA plays an important role in REM sleep generation.

Most recently, Xi et al. [36] have shown in anesthetized chronic cats that the REM sleep produced with a short latency by carbachol injection in the RPO is blocked by prior or subsequent RPO injection of the GABA_A receptor agonist muscimol. However, injection of scopolamine, a muscarinic receptor antagonist, does not block induction of REM sleep by the GABA_A receptor antagonist bicuculline. Those authors concluded that the excitatory cholinergic control of RPO neurons responsible for REM sleep generation is gated by a pontine GABAergic system that exerts its effect postsynaptically by inhibiting RPO neurons, thus resulting in the suppression of REM sleep and the appearance of wakefulness. In the absence of the barrier provided by this GABAergic mechanism, REM sleep emerges [36]. This barrier would exert its effect postsynaptically by a direct GABAergic inhibitory control of REM “on” vRPO neurons supported by the following three facts (1) the abundant GABAergic innervations of vRPO neurons [23, 30]; (2) the scarcity of cholinergic terminals on vRPO neurons [37]; and (3) the frequent observation of convergence onto vRPO neurons by GABAergic terminals in contact with terminals making asymmetric synapses on the same vRPO neurons and probably using excitatory neurotransmitters, such as glutamate [23]. For some

authors [38], the effect of GABA on the actions of acetylcholine in the RPO could take place presynaptically, since activation or blocking of GABA receptors in RPO, respectively, decreases or increases acetylcholine release, suggesting the activation of GABA receptors on cholinergic axon terminals [35]. Presynaptic GABA control of vRPO would be supported by the observation of GABAergic contacting terminals on terminals that make asymmetric synapses; this would allow direct action by the GABAergic terminals on the synapses, thus preventing the release of the corresponding excitatory neurotransmitter [23].

Núñez et al. [39] examined the effects of perifornical hypothalamic area stimulation and local hypocretin application on the electrophysiological activity of vRPO, using extracellular unit recordings in urethane-anesthetized rats. Electrical stimulation of the ipsilateral perifornical area elicited orthodromic responses in vRPO, indicating the existence of an excitatory projection from the perifornical area to the vRPO. However, iontophoretic application of hypocretin-1 through a barrel micropipette in the rat vRPO induced a long-lasting inhibition, which was blocked by prior iontophoretic application of the GABA_A receptor antagonist bicuculline. The authors concluded that the inhibitory effect of hypocretin-1 in the vRPO may be due to the excitatory action of hypocretin on GABAergic interneurons within the vRPO nucleus or to an increase of GABA release from GABAergic afferent terminals, in both cases by the activation of GABA_A receptors. Moreno-Balandrán et al. [13] proposed a similar GABAergic mechanism to explain the significant suppression of REM sleep, without a definitive trend for changes in the other sleep–wakefulness states, produced after the microinjection of hypocretin-1 in the vRPO of freely moving adult cats. Hypocretin-1 from the perifornical hypothalamic region acting at wakefulness-promoting sites, such as the dorsal oral pontine tegmentum, enhances wakefulness [13] while it simultaneously blocks REM sleep generation by increasing inhibitory GABAergic transmission in the vRPO [13].

Watson et al. [40] confirmed the GABA-dependent effect of hypocretin-1 in the RPO using *in vivo* microdialysis and high performance liquid chromatography experiments in anesthetized adult male Sprague-Dawley rats. Administration of hypocretin-1 to the RPO caused a significant, concentration-dependent increase in RPO GABA levels. In addition, RPO microinjection of hypocretin-1 or nipecotic acid (a GABA uptake inhibitor that increases extracellular GABA levels) in unanesthetized rats caused a significant increase in wakefulness and a significant decrease in NREM sleep and REM sleep. Microinjecting 3-mercaptopropionic acid (a GABA synthesis inhibitor that decreases extracellular GABA levels; 3-MPA) into the RPO caused a significant increase in NREM sleep and REM sleep and a significant decrease in wakefulness. They concluded that an increase or a decrease in RPO GABA levels caused an increase or decrease, respectively, in wakefulness. Hypocretin-1 may promote wakefulness, at least in part, by increasing GABAergic transmission in the RPO. The different results obtained in the sleep–wakefulness states after the injection of hypocretin-1 in the rat RPO by Watson et al. [40] (increased wakefulness and decreased NREM and REM sleep) and in the cat vRPO by Moreno-Balandrán et al. [13] (only decreased REM sleep) may be due to species differences or to the different location of the injection, since the latter authors report

increases in wakefulness and decreases in REM and NREM sleep after microinjections of hypocretin-1 in the dorsal oral pontine tegmentum. The volume of the microinjected solutions (100 nl in the rat and 20–30 nl in the cat experiments) and the relative size of the pontine tegmentum in rats versus cats are certainly decisive for appreciating that the structures affected by the diffusion of the injected solution were quite different in the two species.

5 Final Considerations

The results on the location, origin, and role played by the inhibitory neurotransmitter GABA in the vRPO nucleus described in previous sections allow us to, in this last section, consider the role of GABA in the generation, maintenance, and suppression of REM sleep in three aspects:

- Whether GABA inhibits vRPO REM “on” neurons directly or indirectly from brain structures related with the organization of NREM sleep and wakefulness.
- Whether inhibition of GABAergic transmission in the RPO contributes to the generation of REM sleep.
- Whether GABA release can be increased by excitatory afferents.

5.1 Whether GABA Inhibits vRPO REM “on” Neurons Directly or Indirectly from Brain Structures Related with the Organization of NREM Sleep and Wakefulness

Taken together, the previous data indicate that the activity of vRPO neurons is significantly controlled by inhibitory GABAergic terminals. GABAergic afferents essentially originate in diencephalic structures related with the organization of wakefulness and NREM sleep; consequently, through symmetric synapses, they may inhibit the vRPO REM “on” neurons, and the terminals that excite these neurons, during these phases of the sleep–wakefulness cycle. Afferents from the wakefulness and NREM sleep diencephalic structures that use excitatory neurotransmitters and form asymmetric synapses on vRPO GABAergic and serotonergic neurons would also inhibit REM “on” neurons through the enhancement of inhibitory output projection by vRPO GABA and 5-HT neurons (Fig. 3, label 6a) [23, 30]. These ultrastructural findings regarding GABAergic afferents and neurons in the vRPO explain the results of in vitro and in vivo experiments demonstrating that these neurotransmitters inhibit vRPO neuron activity and, thereby, REM sleep [10, 33].

The main source of GABAergic projections to the vRPO is the ipsilateral reticular thalamic nucleus, which provides over 50% of the GABAergic diencephalic afferents to the vRPO [31] (Fig. 4). The reticular thalamic nucleus is

considered to be responsible for sleep spindle generation, a landmark bioelectric component and indicator of the NREM sleep state, chiefly phase 2 NREM sleep in the sleep–wakefulness cycle [2, 41, 42]. Therefore, the inhibitory effect of the reticular thalamic nucleus GABAergic projections to the vRPO might contribute to the suppression of REM sleep induction during the NREM sleep phase of the sleep–wakefulness cycle.

It is known that, in the healthy young adult human, episodes of REM sleep always happen after a period of 70–80 min of NREM sleep, which most of the time follows the NREM sleep phase 2 (Fig. 7a), and, usually, phase 2 resumes immediately upon completion of a REM sleep episode. Consequently, during phase 2 of NREM sleep, in which the reticular thalamic nucleus plays an important role, the prelude to the REM sleep phase may be decided by the suspension of the reticular thalamic GABAergic inhibitory activity on vRPO neurons. This seems to be the last step in homeostatically decreased NREM sleep vitality, although it is not sufficient to produce wakefulness. We should never forget the controlling influence of the suprachiasmatic nucleus, together with the afferent connections to the vRPO from other prosencephalic, diencephalic, brainstem, cerebellar, and spinal cord structures in these mechanisms (Fig. 2). In healthy young cats, the REM sleep episode always follows a long period of NREM sleep, and although NREM sleep most often follows REM sleep, there may also be a period of wakefulness following REM sleep (Fig. 7b). In the latter case, as sometimes happens in humans, the homeostatically decreased NREM sleep will continue its way to wakefulness (Fig. 7).

A GABAergic inhibitory effect on the vRPO similar to that exerted by the reticular thalamic nucleus may be attributed to the GABAergic projection from the zona incerta to vRPO (Fig. 4) [31]. The zona incerta has the same embryological origin as the reticular thalamic nucleus [43]; the zona incerta has been demonstrated to exert a GABAergic inhibition of thalamic projection neurons during NREM

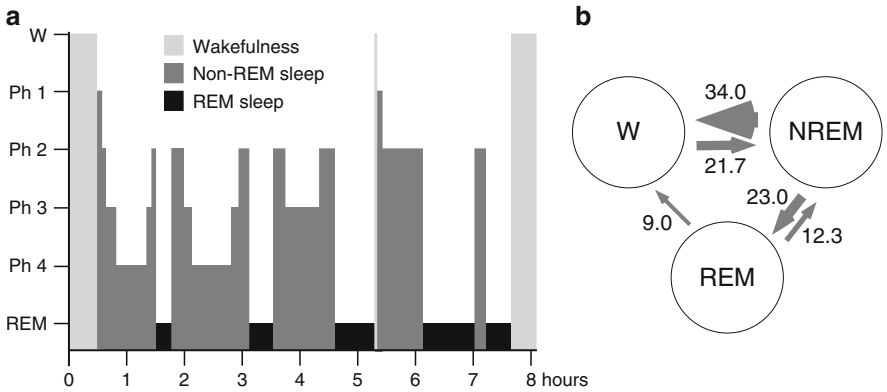


Fig. 7 (a) A night hypnogram of a young man. (b) Mean percentage values of transitions from one state of the sleep–wakefulness cycle to another (relative to the total number of transitions) during 3-h recordings (from 10 to 13 h) in a group of ten adult cats. (b) Done with the data provided by [13]

sleep [44]; an equivalent inhibitory mechanism may be exerted by the zona incerta GABAergic neurons to regulate the vRPO neurons during NREM sleep [31].

According to Rodrigo-Angulo et al. [31], equivalent inhibitory effects may be wielded by the GABAergic projections from the dorsocaudal hypothalamic nucleus, and dorsal and lateral hypothalamic areas to the vRPO that could contribute to the suppression of REM sleep triggering during wakefulness, since these latter structures have been related to the maintenance of wakefulness and a large proportion of their neurons are most active during wakefulness [45–48]. Results that are coherent with those of De la Roza et al. [28] show that about 26% of terminals labeled after anterograde tracer injections into the posterolateral hypothalamus are GABAergic and form symmetric synapses with vRPO neurons.

Also, GABAergic neurons located in midbrain and oral pontine tegmentum that project to vRPO may contribute to the suppression of REM sleep during wakefulness. Using c-fos immunocytochemistry as a functional marker of neuronal activity, Xi et al. [34] found a group of GABAergic neurons within the lateral part of the RPO that is activated during the waking state. In any case, the intrinsic organization of the GABAergic synapses in the vRPO and the different origins of the GABAergic vRPO terminals help explain how GABA could have different effects on the vRPO neural circuits at different phases of the sleep–wakefulness cycle (Fig. 3) [23].

It appears to be that the activity of GABAergic terminals on the vRPO during wakefulness and NREM sleep dominates the influence from excitatory terminal activity, and so, the REM “on” neurons are inhibited. It seems as though the pontine GABAergic system acts within the context of a “gating” mechanism in the RPO to control the generation and maintenance of REM sleep [36]; consequently, REM sleep occurs when GABAergic transmission in the vRPO is suppressed or does not exist; in this situation, REM “on” neurons are activated by excitatory (glutamatergic, cholinergic, etc.) terminals originating in structures related to REM sleep activity. But our ultrastructural results [23, 30] showed that GABA-IR terminals made symmetric synapses or they were apposed to putative excitatory terminals on vRPO REM “on” neurons; thus, GABA may exert its effect on these neurons presynaptically by controlling excitatory neurotransmitter release from the terminals that make asymmetric synapses on vRPO neurons (Fig. 3, labels 1a–1c and 6) [30]. This presynaptic GABA effect has been proposed, as mentioned in the previous section, in cholinergic terminals [38], suggesting that GABA would inhibit REM sleep by inhibiting acetylcholine release in the RPO and indicating that GABA may exert its action not only postsynaptically but also presynaptically, in the latter case by preventing acetylcholine release from cholinergic terminals.

In a more extensive and recent study by the same group [35], the effects of the administration of the GABA_A receptor antagonist bicuculline on acetylcholine release were examined in anesthetized and unanesthetized cats; they concluded that acetylcholine release in the RPO is modulated by GABA_A receptor activation and this effect is consistent with the theory that inhibition of GABAergic transmission in the RPO contributes to the generation of REM sleep in part by increasing pontine acetylcholine release. The authors indicate that the GABA_A receptors that modulate acetylcholine release could be localized presynaptically on cholinergic

terminals or postsynaptically on pontine reticular formation neurons. They conclude (1) GABA levels in the RPO are significantly higher during wakefulness than during REM sleep, and (2) increasing acetylcholine release in the pontine reticular formation is one mechanism by which blockade of pontine GABA_A receptors enhances REM sleep.

The authors [35] have not yet investigated the effect of GABA on any other neurotransmitter, but a similar suggestion about the role of GABA as a both pre and postsynaptic modulator in vRPO could possibly be reached with regard to other neurotransmitters that excite the vRPO REM “on” neurons and collaborate in the induction and maintenance of REM sleep, for instance, glutamate. It has been demonstrated in the cat that microinjections of cholinergic drugs and glutamate on the vRPO trigger the generation of REM sleep [1, 7]. In rat brain-slice preparations, glutamate, like acetylcholine, evokes depolarizations and spike firing in vRPO neurons [32, 33]. Electrical stimulation of the cat pedunculopontine tegmental nucleus, which supplies acetylcholine to the vRPO, evoked orthodromic responses in vRPO neurons, responses, which were blocked by the muscarinic receptor antagonist atropine [49]. Electrical stimulation of the contralateral vRPO, the main source of vRPO glutamate, elicited responses in the vRPO neurons that were blocked by the non-NMDA glutamatergic receptor antagonist CNQX [49]. The deep cerebellar nuclei are another glutamate source, with a rich glutamatergic projection to the vRPO through the superior cerebellar peduncle [1]. Neurotoxic lesions in the cat pedunculopontine and laterodorsal tegmental nuclei decrease REM sleep, as also occurs after superior cerebellar peduncle diathermo-coagulation and, of course, after vRPO lesion [14, 50]. Obviously, GABA may modulate the action exerted by these glutamatergic terminals the same as it does with the action exerted by cholinergic terminals.

5.2 Inhibition of GABAergic Transmission in the RPO Contributes to the Generation of REM Sleep

On the other hand, the GABAergic afferents contacting vRPO serotonergic (which also inhibit the vRPO REM “on” neurons [33]) and GABAergic neurons and terminals and other terminals that form asymmetric synapses with these GABA and serotonergic neurons suggest a mechanism that may assist in the generation of REM sleep by disinhibiting the REM “on” neurons (Fig. 3, labels 2, 4a, 4b, 1c) [30]. This mechanism may consist of the RPO GABAergic neurons that remain active during the REM sleep phase or it may originate in the GABAergic neurons that are most active in the brainstem or diencephalon during natural REM sleep or a REM sleep rebound after deprivation, i.e., the neurons in the dorsal mesopontine tegmentum. In the rat, the percent of REM sleep recovery after deprivation was positively correlated with the number of GABAergic c-Fos1 cells that were next to monoaminergic neurons in the latter region [51]. This supports the hypothesis that

GABAergic neurons are active during REM sleep and could thus be responsible for inhibiting neighboring monoaminergic neurons; inhibition of monoaminergic neurons may be necessary for the generation of REM sleep [51]. On the other hand, GABAergic neurons of the dorsal mesopontine tegmentum in the cat express c-fos during carbachol-induced REM sleep and it has been proposed that they may participate in the regulation of REM sleep, just as the dorsal raphe nucleus GABAergic neurons participate in the inhibition of serotonergic neurons that occurs during natural REM sleep [52, 53]. In addition, the results of microinjections of muscimol and bicuculline in the pedunculopontine nucleus suggest that pedunculopontine GABAergic neurons act on GABA_A receptors within this nucleus to facilitate the generation of REM sleep by suppressing the activity of waking-related processes within the nucleus [54]. Also, the possible vRPO afferents from these dorsal mesopontine GABAergic neurons may be part of the mechanism that through disinhibition of REM “on” vRPO neurons could assist in the generation of REM sleep in vRPO [30]. Likewise, GABAergic neurons in RPO may be active during NREM sleep and inactive during REM sleep [55]. This would provide local GABAergic inhibition of vRPO REM “on” neurons during NREM sleep and wakefulness and would disinhibit them during REM sleep. This is quite consistent with the finding that microinjection of GABA_A receptor antagonist bicuculline in RPO enhanced REM sleep and suggests that the mechanism of action could be through an antagonization of local GABAergic inhibitory neurons and terminals. It is very interesting that GABA might contribute to the induction and maintenance of REM sleep by inhibiting serotonergic and GABAergic neurons in the vRPO; however, it is also possible that GABAergic afferents contacting vRPO 5-HT-IR and GABA-IR neurons and terminals might arise from other brain area neurons that are active during REM sleep. In this case, inhibition of vRPO serotonergic and GABAergic vRPO neurons (probably REM “off” neurons) and terminals would disinhibit REM “on” neurons through GABAergic and serotonergic mechanisms in a way that would be important for the generation of REM sleep [30].

5.3 Increase of GABA Release by Excitatory Afferents

On the other hand, GABAergic vRPO neurons and terminals may change the excitatory effect of inputs from other brain structures into an inhibitory effect over vRPO REM “on” neurons [30]. For example, Rodrigo-Angulo et al. [31] have shown that basal forebrain and diencephalon structures closely related with NREM sleep and wakefulness send abundant non-GABAergic projections, possibly using excitatory or inhibitory neurotransmitters, to the vRPO. The authors suppose that some of these projections, principally those originating in the lateral-posterior hypothalamus [28], may inhibit vRPO REM “on” neurons, mainly during wakened periods of the sleep–wakefulness cycle, whether directly through symmetric synapses (Fig. 3, label 7) or indirectly through the activation of local GABAergic or serotonergic vRPO neurons (Fig. 3, label 6a), as has been proposed to occur with

the hypocretinergic projections from the lateral-posterior hypothalamus in the rat [39]. Actually, hypocretin-1 produces a GABA-mediated inhibition in vRPO neurons [39]. The hypocretinergic projection to the vRPO [39] may be part of the fibers originating in the posterolateral hypothalamus that make asymmetric excitatory-type synapses with different vRPO neurons [28]; these fibers may activate inhibitory GABAergic- and serotonergic-vRPO cells and, consequently, could generate postsynaptic inhibitory processes within the vRPO (Fig. 3, label 6a). However, any inhibition of vRPO neurons would also probably be due to presynaptic GABA release from GABAergic terminals apposed to an asymmetric hypocretinergic terminal on the same dendritic segment of the vRPO neuron (Fig. 3, labels 1a, 1b, 1d) [13, 23, 28, 30]. This mechanism is akin to that previously proposed for hypocretinergic activation of GABAergic terminals in the tuberomammillary nucleus [56]. Consequently, it appears that hypocretin-1 gives rise to increased GABA secretion in the RPO, as has been demonstrated by the observation that dialysis administration of hypocretin-1 to the rat RPO produced a statistically significant, concentration-dependent increase in RPO GABA levels [40].

The correct interpretation of the results of microinjection experiments in the pontine tegmentum requires considering not only species differences vis à vis anatomy, the size of the brainstem structures, and REM sleep regulating mechanisms, but also, and most importantly, the precise location and volume of the microinjection. If the increased GABA release brought about by hypocretin microinjections is confined to the vRPO, it will provoke a specific decrease of REM sleep in the cat [13], but if the microinjection covers a wide area of the RPO in the rat or is confined to the dorsal oral pontine tegmentum in the cat, it will significantly increase wakefulness with an associated decrease in both NREM and REM sleep [13, 40]. These results are consistent with the facts that the dorsal mesopontine tegmentum is a pivotal structure in the reticular activating system and that the vRPO is an essential structure for the induction and maintenance of REM sleep. Consequently, hypocretin excites the dorsal mesopontine tegmentum-waking structures and directly inhibits the vRPO structure responsible for REM sleep [13]; what is more, the accompanying increase in GABA release has an important role in both phenomena.

Acknowledgments This work was supported by Grants BFI2002-01314, BFU2006-07430 and BFU2009-06991 from Spanish Ministry of Education and Science. We acknowledge Ms. Carol F. Warren for her assistance in reviewing English language usage.

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The Role of GABAergic Modulation of Mesopontine Cholinergic Neurotransmission in Rapid Eye Movement (REM) Sleep

Gerald A Marks and Christopher M Sinton

Abstract The complexity of the pontine neuronal mechanisms that control and modulate expression of rapid eye movement (REM) sleep has been only recently recognized. In this review of some of these mechanisms, results from studies in the cat and rat are summarized and compared, and the differences between neuronal populations that might be effective, modulatory, or executive for REM sleep control are considered. One of the principal pontine areas that has been found to be important for the induction of REM sleep is located in the nucleus pontis oralis. Another is located in a more dorsocaudal area. The latter, designated the peri-locus coeruleus α in the cat, corresponds most closely with the sublaterodorsal (SLD) nucleus in the rat. In both areas in the cat, cholinergic mechanisms are important for REM sleep induction, whereas in the rat, only the pontis oralis area is cholinergically sensitive. In contrast, the SLD is responsive to glutamatergic processes. The emerging recognition of the importance of GABAergic modulation of these sites in both species is reviewed, and from this work, some preliminary conclusions can be drawn. In the caudal pontis oralis of rat, GABAergic mechanisms can affect the release of acetylcholine, inferring that transmitter release in a terminal region can be decoupled from impulse flow. This is important in light of the concept of changes in the discharge rate of reciprocally interacting populations of neurons being correlated with differences in vigilance state. A second conclusion, based on the work in the SLD, is that network interactions are critical for REM sleep control. The latter can be extended to conclude that activation of neurons that are effective

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for at least two of the signs of REM sleep may be sufficient to induce the state. Distributed networks would obviate the need for a single locus of executive control for REM sleep. But another possibility derived from the SLD studies is that executive control of REM sleep may actually represent the coordination of effective mechanisms, explaining the difficulty that has been encountered in finding a REM sleep “generator.”

1 Introduction

Rapid eye movement (REM) sleep is dependent on a set of anatomically distributed neural mechanisms. To understand these mechanisms, it is necessary to identify the individual components and determine the manner in which they interact. This has proven to be a challenging process. Historically, concepts of localization of brain function drove a quest to find the REM sleep “generator” utilizing the limited number of tools that were available at the time, primarily relying on lesion techniques. To this day, however, no discrete focal lesion has resulted in the discovery of any single mechanism found to be necessary and sufficient for the production of REM sleep. Furthermore, initial attempts at modeling multiple interactive mechanisms proved inadequate primarily due to a limited knowledge of the number of mechanisms involved. Use of a variety of recently developed, more powerful methods has revealed several new mechanisms and their interactions: this knowledge may bring us closer to a more complete elucidation of the way that the expression of REM sleep occurs.

In this chapter, we focus on those REM sleep mechanisms that are localized to the pons and use acetylcholine (ACh) and γ -aminobutyric acid (GABA) as neurotransmitters. The emphasis is on specific REM sleep induction areas that have been identified in the cat and rat. Current knowledge is reviewed from a neuroanatomical and neurochemical perspective, but the quest to identify the mechanisms of REM sleep generation remains incomplete. The convergence of findings over many years and in two species, however, add support to our expectation that future work will underline the importance of the mechanisms that we describe here.

2 Categorizing REM Sleep Mechanisms

When first discovered in the human, and then later described in the cat, REM sleep appeared to be an extraordinary phenomenon because, although the state was evidently classifiable as sleep, from an electrophysiological perspective, the brain appeared to be very much awake. Thus paralleling the results from the human, independent work in the laboratories of Jouvet and Dement found that REM sleep in the cat could be distinguished by various electrophysiological signs that could be associated with wakefulness, including brain arousal, eye movements, myoclonic twitches, and cardiovascular variability [1, 2]. Brain lesion studies followed in an

attempt to identify the generating region for this state, and Jouvet noted that a complete transection separating the pons from the midbrain permitted the periodic appearance of the signs of REM sleep caudal to the transection. However, these signs were completely absent in recordings from the isolated forebrain [2]. This finding was interpreted as indicating that the brain structures sufficient to produce REM sleep lay in the region of brain caudal to the transection, including the pons and medulla, and that structures rostral to the transection were not necessary for production of the state.

However, conflicting results from subsequent studies by other groups were reported when transections were made that separated the pons from the medulla oblongata. Such lesions were found to result either in no identifiable signs of REM sleep on either side of the lesion [3, 4] or a periodic confluence of the critical signs of REM sleep in brain segments rostral to the transection [5]. The issue of whether mechanisms in the pons, alone, are sufficient for the production of REM sleep therefore remained unresolved, as it remains today. Indeed, ample evidence supports mechanisms rostral to the pons as capable of altering the expression of REM sleep and thus should also be considered, at a minimum, as being involved in REM sleep control. For example, focal lesions of the suprachiasmatic nucleus of the hypothalamus disrupt the normal pattern of REM sleep expression over the nycthemeron [6]; cats with rostral pontine transections do not exhibit a rebound following an instrumental REM sleep deprivation [7]; and other hypothalamic lesions affect homeostatic control with a similar lack of rebound [8]. Furthermore, REM sleep expression is subject to modulation by a host of factors including, but not limited to: time of day, recent past expression of the state, lighting, ambient temperature, learning, and various forms of psychological and physical stressors. Components of these REM sleep-modulating mechanisms are distributed throughout the brain.

Leading to the discovery of the state and as observed in the earliest studies, REM sleep is a convergence of many apparently distinct processes. These processes are tonic, appearing continuously throughout the entire REM sleep episode, and phasic, which are intermittent events occurring sporadically during the episode. The constellation of events present in REM sleep include: high-frequency, low-voltage activity in the electroencephalogram (EEG); theta rhythm (6–8 Hz) in the hippocampal EEG; atonia of the postural musculature; rapid eye movements; phasic field potentials, pontine-geniculate-occipital (PGO) waves, recorded at different locations along the neuraxis; myoclonic twitches; and cardiorespiratory variabilities. Each of these indices can be selectively affected by various neurophysiological manipulations indicating not only, at some level, an independence of the mechanisms that subserve them [9] but also that, individually, these signs are not necessary for the appearance of the state. Although the functional significance of the individual processes remains obscure, all signs are normally present in every REM sleep episode. This infers the existence of an executive mechanism for the generation of REM sleep. Although the nature of this executive mechanism is unknown, it is also likely to be comprised of several distributed and interacting systems.

To the extent that it may be heuristically valuable to categorize REM sleep mechanisms as those that are modulatory, effective, or executive, such functional distinctions, with respect to any individual mechanism, are not necessarily mutually exclusive. Components of the executive must perform the direct recruitment of effective elements, and the same or additional components of the executive are subject to modulation. There is also the distinct possibility that an effective component could feed back onto modulatory influences. The challenge to understanding such a system lies in gaining a basic knowledge of the functioning of complex interactions in neural networks. One approach to understanding these mechanisms has been to identify the individual components and determine the manner in which they interact.

3 Pontine Areas for REM Sleep Generation

In the pons, there are regions in which the local application of a variety of agents, including neurotransmitter receptor ligands, can induce a REM sleep-like state at short latency. These areas are known as REM sleep induction areas. The ability of pharmacological agents, interacting with brainstem mechanisms to alter significantly the expression of the state, strongly implicates these regions in the natural regulation of REM sleep. Under certain conditions, the effect of triggering REM sleep, with all its signs, is consistent with the activation of an executive mechanism. The finding that neurotransmitter receptors can mediate these effects indicates a basis for interaction when the source of the natural ligand for the receptors is not local. Thus, a study of the REM sleep induction areas is a logical choice to pursue identification and interaction of the mechanisms of REM sleep generation.

Although not necessarily with the same rationale that is presented here, a study of REM sleep induction areas has a long history in investigations of sleep mechanisms. Like other lines of inquiry, faulty interpretation of data occurred that then tended to be corrected. There are, however, several issues that remain unresolved. One is the result of transecting the brain at the pontomedullary border, discussed above (cf. Sect. 2). This issue has a significant impact on theorizing about pontine REM sleep induction areas. Procedural differences could account for variance in the results from different investigators, but there is also the possibility of differences in the experimental subjects. Most of what is known about mechanisms of REM sleep comes from studies on cats and rats. Despite great similarity in the expression of REM sleep between these species, significant differences exist in the results from procedures investigating REM sleep induction areas. The basis of these species differences is unknown. One continuing source of confusion lies with the different neuroanatomical nomenclature typically used in the cat and rat for this region. Another potential source of confusion is a real difference in physiology. Although these differences could and indeed should be trivial to the functioning of the system, interactions with experimental brain lesions or drug injections often produce significantly different results. The outcome is that experimental findings derived from one species cannot necessarily be applied

to another. Until these issues are resolved, data should be interpreted with respect to its source. This line of reasoning will be followed here.

In this review, we emphasize cholinergic mechanisms because evidence to date supports an important role for ACh in REM sleep control. But interestingly, whether cholinergic mechanisms are actually necessary for the appearance of REM sleep remains an open question. Excitotoxic lesions in the cat aimed specifically at the pedunculopontine tegmentum (PPT), the region of cholinergic cell bodies projecting throughout the neuraxis and especially the pons (cf. Sect. 4.2), resulted in reductions in REM sleep. These reductions lasted for at least 3 months and were correlated with the number of cholinergic neurons that were lost. Reductions in REM sleep were also correlated with the size of the lesions [10]. Inasmuch as the largest and most effective lesions included structures outside the PPT, it remains unclear how much the behavioral effects of the lesions were dependent on destruction of noncholinergic cell groups. Development of a selective neurotoxin for mesopontine cholinergic neurons would enable this question to be answered.

4 REM Sleep Induction Areas in the Brainstem of the Cat

4.1 *Short Latency Cholinergic-Sensitive REM Sleep Induction Areas*

Shortly before the discovery of the widely projecting mesopontine cholinergic system [11], it was found that local intracerebral injections of agents potentiating cholinergic transmission in the pontine reticular formation (PRF) of the cat could trigger, at short latency, a state indistinguishable from natural REM sleep [12]. This finding was consistent with a concept of localized function and spurred interest in searching for a generator mechanism. Based on the effectiveness of the cholinergic receptor agonist carbachol to induce REM sleep in the cat, certain pontine regions were reported to be the locus of REM sleep induction. These areas included the dorsal nucleus pontis oralis (PnO), the ventral PnO, and the peri-locus coeruleus α (peri-LC α) [13] (Fig. 1). In the first systematic investigation, Baghdoyan and colleagues [14] described an area that produced REM sleep with a minimal latency to onset. This area, inferred from the anatomical distribution of injection sites, was an area in the rostradorsal PnO at the level of the ventral tegmental nucleus of Gudden (VTg). Shortly thereafter, independent investigations supported another induction area lying more caudal in the dorsal pons in the region ventral to the locus coeruleus (LC) named the peri-LC α [15]. Subsequently, these two areas were linked in a rigorous mapping study describing a “short latency axis”, which approximated a cylinder extending in the sagittal plane encompassing the two sites [16].

Partly based on the findings of a latency gradient for carbachol induction of REM sleep by injections outside the identified axis, Yamamoto and colleagues [16] proposed a collection of distributed mechanisms in the region and the differential

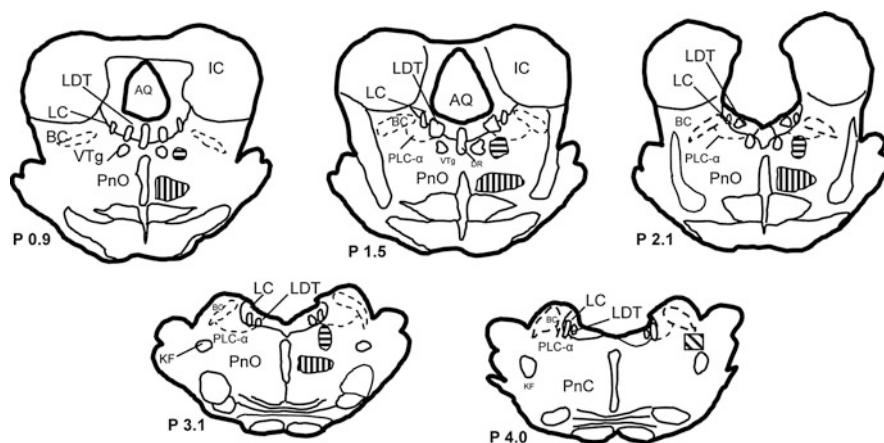


Fig. 1 Schematic drawings of coronal sections through the pons of the cat illustrating some regions important for the induction of REM sleep. The area delineated by the *horizontal lines* corresponds to the “axis of short-latency” for carbachol induction of REM sleep [16], and the area delineated by the *vertical lines* is the ventral PnO REM sleep induction area [13]. Also shown, as an area delineated by the *box* containing lines at an angle, is the region of the putative PGO wave generator site [19]. Indicated below each section is the coordinate (in millimeters) of the coronal plane of the section posterior to interaural zero. AQ aqueduct; BC brachium conjunctivum; DR dorsal raphe nucleus; IC inferior colliculus; KF Kölliker-Fuse nucleus; LC locus coeruleus; LDT laterodorsal tegmentum; PLC- α peri LC- α ; PnC caudal pontine reticular nucleus; PnO oral pontine reticular nucleus; VTg ventral tegmental nucleus

effect on multiple neuronal populations through diffusion of the carbachol from the injection site. Thus, injections within the axis of short latency are optimal to influence a critical number of mechanisms, but those injections outside this region produce less optimal effects and can result in the appearance of only some of the signs of REM sleep or arousal [17]. The dorsal pons is the locus for effective mechanisms responsible for many of the signs of REM sleep. Several lines of evidence, for example, support the LC α and peri-LC α as critical for the generation of REM sleep muscle atonia [18]. And a region lateral to the LC α , in the peribrachial area, has been implicated in originating PGO wave activity [19] (cf. Fig. 1). Thus, studies injecting cholinergic agonists into this region in the cat have reported induction of a dissociated state characterized by muscle atonia with PGO waves against background signs of wakefulness (e.g., [17]).

More recently, injections of carbachol in the nanoliter range have been used to identify a large region in the ventral PnO that supports the induction of REM sleep at short latency [20]. This area extends to the full anterior–posterior extent of the more dorsal “axis” of Yamamoto and colleagues [16], indicating that the distribution of cholinceptive REM sleep-promoting sites in the cat appears to be more extensive than originally determined. This is consistent with a distributed system, which can be activated at a great variety of spatial locations. Importantly, disruption of the system is more difficult. Small focal lesions in the pons are unable to

eliminate REM sleep completely, but rather result in disassociating the convergent physiological signs normally always present in each REM sleep episode [21].

4.2 The Source of Cholinergic Innervation to the REM Sleep Induction Areas

The spatial distribution of cholinergic fibers and terminals in this pontine region is complex. Furthermore, since the release of ACh during different vigilance states is controlled by specific neuronal mechanisms, selective targeting of various muscarinic receptors in the region may occur. This is therefore unlike the direct, widespread, and undifferentiated effect of an intracerebral injection. Based on studies with retrograde tracers, neurons providing the source of ACh to the region were localized to the mesopontine cholinergic nuclei, the laterodorsal pontine tegmentum (LDT) and the PPT (Figs. 1 and 2), and to a lesser degree, sites in the medulla [22–25]. One specialization found only in the cat is the addition of an immunologically identified distribution of cholinergic neurons in the medial and lateral peribrachial area, as well as in the LC α and peri-LC α [26]. Most of the putatively identified mesopontine cholinergic neurons discharge at their highest rates during REM sleep and wakefulness, but a subset selectively discharges during REM sleep [27, 28]. Microdialysis studies in the PRF have shown that ACh levels in this area are significantly greater in REM sleep than in non-REM (NREM) sleep and wakefulness [29]. This led to the hypothesis that the REM sleep active (i.e., REM-on) cholinergic neurons project to the region of the REM sleep induction area.

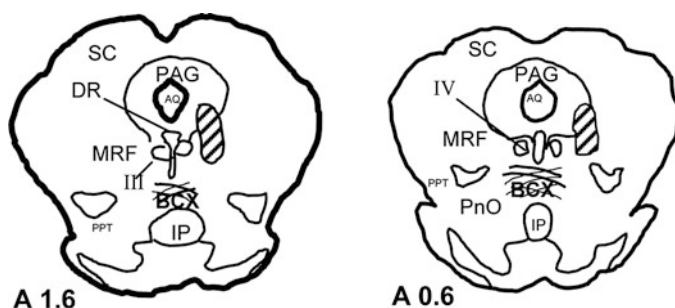


Fig. 2 Schematic drawings of coronal sections through the region of the pontomesencephalic junction of the cat to illustrate a region from which a GABAergic innervation of the REM sleep induction areas of the pons originates. This region, delineated by the *angled lines*, is that in which local muscimol injections induce REM sleep [48]. Indicated below each section is the coordinate (in millimeters) of the coronal plane of the section anterior to interaural zero. *III* oculomotor nucleus; *IV* trochlear nucleus; *BCX* decussation of the brachium conjunctivum; *IP* interpeduncular nucleus; *MRF* mesencephalic reticular formation; *PAG* periaqueductal gray; *PPT* pedunculopontine tegmental nucleus; *SC* superior colliculus. For other abbreviations, see legend to Fig. 1

4.3 The Interaction of REM-on and REM-off Neuronal Populations

The hypothesis of cholinergic REM-on cells projecting to a REM sleep induction area parallels the conclusions that were drawn from some early electrophysiological studies, though when first formulated, the identities of the relevant cell populations were unknown. McCarley and Hobson [30] had noted that the change in the discharge rate of neurons over the sleep cycle in the LC area of the cat was the inverse of that of neurons in the gigantocellular tegmental field (FTG), corresponding to the most caudal PRF and rostral bulbar reticular formation [31]. Thus, LC area neurons were found to decrease their discharge rate before the onset of REM sleep whereas FTG neurons increased in discharge rate. The FTG lies in the caudal pons and thus within the area that Jouvet [2] had defined as encompassing the brain structures sufficient for REM sleep. This led to McCarley and Hobson [30] proposing two mutually interacting and inhibiting populations of REM-on and REM-off neurons and the development of a reciprocal interaction model to describe their changes in discharge rate. Indeed, the FTG REM-on area was considered the locus of the elusive REM sleep generator at the time [32]. This and several other aspects of the model were considered controversial for many years, but it is noteworthy that the basic concept of mutually interacting populations of REM-on and REM-off cells has proved heuristically valuable. When first formulated, the neurochemical identities of the relevant cell populations were unknown. More critically, the FTG cell field was later shown to be largely comprised of cells that are not specifically responsive to changes in the sleep cycle, but rather reflect muscle activity. Subsequently, however, a group of REM-on cells was identified as the subset of the mesopontine cholinergic neurons described above. Furthermore, the REM-off cells in the LC region could only be tentatively identified at the time the model was formulated as being noradrenergic (NE), and in fact it was several years later that their neurochemical identity was confirmed.

The model has therefore been adapted as new results have become available to allow its refinement and improvement. This process began with the neurochemical identification of the relevant cholinergic and monoaminergic cells groups, extending the latter to include the serotonergic (5-HT) cells of the dorsal raphe nucleus. Results over many years have supported the concept of mutually inverse discharge rates of two neuronal populations. For example, the inhibition of the REM-on cholinergic neurons by REM-off monoaminergic neurons has only been demonstrated relatively recently. The first studies that showed that 5-HT directly inhibits a subpopulation of LDT/PPT cholinergic neurons [33–35] were conducted *in vitro*. Subsequently, it was shown that the effect of NE on these LDT/PPT cholinergic neurons was also inhibitory [36]. Moreover, noncholinergic interneurons, presumably GABAergic interneurons, are excited by NE [37]. Since GABAergic interneurons would inhibit cholinergic neurons, this provides another mechanism of inhibition of cholinergic LDT/PPT neurons by NE. Hence the influence of GABAergic neurons in the REM-on cell area is critical for the mutual interaction

that correlates with the onset of REM sleep. Since, as we discuss below (cf. Sect. 4.5), GABAergic mechanisms are also important in the REM sleep induction regions, GABA may play a central role in the control of REM sleep. Indeed, recent data have added additional complexity to this concept of REM-on/REM-off neuronal interactions, because it is now apparent that presynaptic GABAergic mechanisms in the REM sleep induction area can locally inhibit cholinergic innervation during REM sleep (cf. Sect. 5.3). This would decouple the cholinergic influence from impulse flow and allow modulation from multiple influences. The interaction of REM-on and REM-off neuronal populations, each with widespread projections, was originally considered a potential candidate for an executive control mechanism of REM sleep. But with greater understanding of the interactions between these cell populations and the inclusion of GABAergic mechanisms, the concept is currently better considered as reflecting the modulatory and effective aspects of REM sleep control.

4.4 Cholinergic Action in the REM Sleep Induction Areas

Cholinergic neurons are inhibited by cholinergic agonists acting at muscarinic autoreceptors, resulting in a reduction of ACh release [38]. Therefore, the sites of action for REM sleep induction following the injection of cholinergic agonists are presumably noncholinergic neurons. For example, the peri-LC α is one site that contains putative noncholinergic REM-on neurons that are excited by carbachol [28]. The use of relatively selective muscarinic antagonists has shown that cholinergic REM sleep induction at this site is probably mediated through the m3 muscarinic receptor subtype [39], which is associated with an excitatory action [40]. In the more dorsal aspects of the induction area, however, mediation is likely to be through the m2, or possibly m4, receptor subtypes [41], which are associated with an inhibitory action [40]. The cholinergic receptors mediating effects in the ventral PnO have not been explored. It is likely therefore that cholinergic REM sleep induction in the pons includes both excitation and inhibition of multiple and selected neuronal populations. Such a complex pattern of effect could therefore include action at the effective neurons for REM sleep as well as at putative executive neurons.

4.5 GABAergic Mechanisms in the REM Sleep Induction Areas

GABA is the neurotransmitter accounting for most of the rapid inhibitory synaptic transmission in brain through the GABA_A-type ionotropic receptor. GABAergic mechanisms have long been recognized as having a role in promoting NREM sleep, and indeed drugs that potentiate GABAergic neurotransmission comprise the majority of hypnotic agents. An early indication of a role for GABA in REM

sleep was the REM sleep-suppressing action of many of these drugs [42]. Then, Chase and colleagues [43] reported a short latency, REM sleep onset in the cat following microinjection of a GABA_A antagonist, bicuculine (BIC) into the pontine peri-LC α region. This result indicated that GABA exerted a negative control at this site and the withdrawal of its influence therefore produced REM sleep induction. This was an important finding that resulted in the first recognition of GABAergic mechanisms as critical in the control of REM sleep. Indeed, subsequent work has continued to support this view and has led to current conceptions that have shifted the emphasis in REM sleep executive mechanisms from cholinergic to GABAergic.

Like cholinergic agonists, GABA_A antagonists injected into the peri-LC α region induce within minutes a state showing all the signs of normal REM sleep, but with significantly extended episode durations. Unlike the effect of cholinergic agonists, however, the duration of action on REM sleep expression following a single injection is relatively short lived and lasts about 1 h. This is consistent with an effect that depends on receptor occupancy. Injection of a glycine receptor antagonist fails to induce REM sleep, so this effect is specific to GABA [44]. This action of GABA_A antagonists to increase REM sleep may differ between the caudal and rostral aspects of the induction area defined by carbachol. Ventral sites have not been tested. When BIC was dialyzed into the more rostral PnO of the cat, it increased the subsequent time spent in REM sleep rather than triggering the state at short latency [45]. Results from this study also showed that BIC resulted in increased levels of ACh, possibly indicating that the REM sleep augmentation was a consequence of increased ACh release. This is another difference from results obtained in the caudal region of the induction area, where REM sleep induced by BIC is not blocked by the muscarinic receptor antagonist scopolamine [46]. In contrast, scopolamine blocks REM sleep induced by carbachol. Interestingly, and unlike the effects of the antagonists, local injection, at the same loci, of a GABA_A agonist resulted in a period of several hours of wakefulness [43]. However, it is not yet known whether the contrasting actions of the GABA_A agonist and antagonist are at receptors on the same neurons. That is, some neurons in the region may promote wakefulness when inhibited and others may promote REM sleep when disinhibited. The local effects of GABA in the REM sleep induction area are also discussed below in view of recent significant data obtained in the rat (cf. Sect. 5.3).

An important question concerns the source of the REM sleep induction area GABAergic afferents that are capable of inducing wakefulness and, through their blockade, of inducing REM sleep. Local GABAergic interneurons are candidates. Application in the peri-LC α region of antisense oligonucleotide directed towards the isoform of the GABA-synthesizing enzyme, glutamic acid decarboxylase 67 (GAD67), blocked transcription of the enzyme and thus likely reduced GABA synthesis in the local GABAergic interneuron population [44]. This procedure resulted in significantly augmenting REM sleep for several days, the probable duration of the resulting knock-down in GABA synthesis. However, GAD67 transcription might be affected in GABAergic neurons that are not local since it is feasible that the oligonucleotide was taken up by terminals and then retrogradely

transported to cell bodies of nonlocal GABAergic neurons. Alternatively, if GAD67 is transcribed in the terminals, this transcription could be affected. Hence, this result cannot be taken as definitive proof that the local peri-LC α GABAergic interneurons are responsible for the effect on REM sleep. In fact, evidence also implicates GABAergic neurons with cell bodies in other areas as providing the relevant GABA input. One such area is the ventrolateral periaqueductal gray extending into the mesencephalic reticular formation (MRF). Bilateral lesions of this area [47] and injections of a GABA_A agonist into the region resulted in significant increases in REM sleep [48] (Fig. 2). These results are consistent with the removal of a GABAergic innervation to the peri-LC α and like antagonists, inducing REM sleep. Recent data derived in the rat are consistent with this interpretation and are discussed below (cf. Sect. 5.5).

5 REM Sleep Induction Areas in the Brainstem of the Rat

During the first decades of research into the mechanisms of sleep, the cat was the preparation of choice, but as techniques were refined and miniaturized, it became feasible and economical to study the rat, which has now almost entirely replaced the cat in this work. As in the cat, homologous regions identified as REM sleep induction areas have been found in the rat, though responses to pharmacological agents at these sites differ between rat and cat. Thus, in the caudal aspects of the PnO (PnOc), at the level of the VTg, cholinergic agonists do not trigger REM sleep at very short latency in the rat, but instead induce a significant, long-lasting (ca. 8 h) increase in REM sleep, primarily through an increase in the frequency of REM sleep episodes [49–51]. In both rat and cat, intracerebral injection directly into this area of several different ligands, which do not act at cholinergic receptors, also induces a prolonged increase in REM sleep [52–58]. The nature of this regulation may be through alteration in the propensity to trigger REM sleep, which as noted above (cf. Sect. 2), is subject to a variety of regulatory influences that can affect the expression of the state over prolonged intervals. The PnOc may thus be a region serving to integrate environmental and experiential factors in the expression of REM sleep.

5.1 *The PnOc, a Cholinergic-Sensitive REM Sleep Induction Area*

The cholinergic REM sleep induction area in the rat (i.e., the PnOc) is a circumscribed region of the PnO extending caudally from the decussation of the superior cerebellar peduncle to the border of the PnO with the nucleus pontis caudalis (PnC) (Fig. 3). To date, no evidence for an axis of short latency has been reported nor has

a distinction been drawn between dorsal and ventral aspects. Effective REM sleep induction follows carbachol administration in this area at doses that are lower, in terms of both volume and concentration, than those typically used to induce REM sleep in the cat. In the rat, doses too low are ineffective, but higher doses result in reduced REM sleep and increased wakefulness, thus producing an inverted U-shaped dose response curve. The magnitude of the REM sleep increases are less than in the cat and although the latency to REM sleep onset can be significantly decreased, latencies of greater than 30 min should not be considered a direct triggering of REM sleep. Rather, the nature of this response is consistent with the concept of a drug diffusing to intercalated neuronal populations that both promote and antagonize REM sleep. Hence the different anatomical distributions of these populations in rat and cat could give rise to differing responses when cholinergic agonists are injected into the peri-LC α /PnOc in the two species. The size of the region and the narrow effective dose range for carbachol to induce REM sleep in the rat has led to reported failures to produce the effect [59]. However, with careful placement and correct doses, local injections into the PnOc of the rat result in consistently reproducible effects [49–51, 60].

The prolonged effects, in both cat and rat, of a single injection of a cholinergic agonist indicate that the drug only initiates a process that continues after it has diffused away. This could reflect a cascade of intracellular changes that follow activation of the G-proteins coupled to the muscarinic receptors mediating the response. Such a hypothesis is supported by the induction of REM sleep that follows the injection of an inhibitor of adenylate cyclase (AC) into the PnOc. Indeed, the effect produced by this procedure is almost identical to that produced by cholinergic agonists [61]. Additional support for the involvement of intracellular cyclic AMP (cAMP) comes from the finding that protein kinase A (PKA) activity is reduced in the PnOc of rats sacrificed during the enhanced REM sleep expression that follows deprivation of the state [62]. Since AC is an enzyme that catalyzes cAMP production from ATP and, in turn, the level of cAMP controls PKA activity, these data also provide some indications of the intracellular events in PnOc neurons that are occurring naturally during REM sleep or that are induced by cholinergic activity.

5.2 The Source of Cholinergic Innervation to the PnOc

Interestingly, electrical or glutamatergic stimulation of the cholinergic PPT can induce REM sleep in the rat with a short latency to onset. Presumably the short latency in these studies resulted from a more selective release of ACh than is possible with drug injections. For example, if this action is in the PnOc, the stimulated release of ACh may more effectively target neurons that promote REM sleep. However, the identity of the neurons in the heterogeneous population of the PnOc that promote REM sleep is not yet known. In vitro studies report that about 2/3 of PRF neurons are excited by carbachol, but about 1/3 are inhibited

[63, 64], and recent evidence indicates that a preponderance of inhibited neurons in the mouse are GABAergic [65]. In the rat PnOc, as in the cat rostral pontine REM sleep induction area, carbachol induction of REM sleep is likely to be mediated through m2/m4 muscarinic receptor subtypes [51, 66]. The m2/m4 muscarinic receptor subtypes couple to G-proteins that inhibit AC and are associated with inhibitory effects [67]. However, knockout mice that are null for either or both the m2 and m4 receptors have normal amounts of REM sleep [68], though no cholinergic REM sleep induction or test of homeostatic control was attempted in these animals. Unlike results reported in the cat, there is no significant change in REM sleep in rats following lesions of the PPT. However, lesioned rats require fewer awakenings to deprive them of REM sleep and smaller REM sleep rebounds following the deprivation [69]. This is consistent with a role for ACh as a modulator of REM sleep propensity, and the PnOc may be a major locus of this action.

5.3 The PnOc as an Integrator of Multiple Influences and the Role of GABA

The hypothesis of a central role for the cholinergic system and the PnOc as a site of integration of many REM sleep modulatory influences is supported by the multiple mechanisms that act in the PnOc and depend on the cholinergic system. To date and based on the action of the muscarinic antagonist atropine to block REM sleep induction, the following receptor systems have been shown to require the cholinergic system in the PnOc: A_{2A} adenosine receptor agonists [52], vasoactive intestinal polypeptide [55], pituitary adenylate cyclase activating polypeptide [54], and α_2 adrenergic receptor antagonists [56]. Some mechanisms of REM sleep induction in the PnOc are independent of the cholinergic system, e.g., A_1 adenosine receptors agonists, which are not blocked by atropine [52]. But the multiplicity of mechanisms involving modulation of the cholinergic system in the PnOc underline the importance of the region as a nexus of several influences. Recently added to this list is the action of GABA in the PnOc.

As in the cat, injection of GABA_A antagonists into the PnOc of rat induces REM sleep [58, 70]. This effect is remarkably similar to that of carbachol with a prolonged duration of action, an increase in episode frequency, and modest reductions in latency. Furthermore, the REM sleep-inducing action of the GABA_A antagonist gabazine (GBZ) is blocked by a preinjection of atropine at a dose that, alone, is too low to affect REM sleep [70]. GABA_A antagonists dialyzed into the PnO increase ACh release in the cat [45], a result recently replicated in mouse [71]; and cholinergic terminals in the PnOc express GABA_A receptors [72]. Taken together, these data strongly suggest that GABA in the PnOc presynaptically inhibits the release of ACh. Thus, antagonism of the GABA_A receptors would disinhibit release and result in an atropine sensitive cholinergic induction of REM

sleep. An important implication of these findings is that the cholinergic innervation of the PnOc may not necessarily be from neurons whose activity is restricted to REM sleep to achieve a REM sleep-selective pattern of ACh release. Indeed, many putatively cholinergic PPT/LDT neurons do not have activity patterns selective to REM sleep [73, 74]. Thus, local control of ACh release in the PnOc could be a mechanism by which GABA and other neurotransmitters interact to modulate REM sleep expression.

5.4 The SLD, a Noncholinergically Sensitive REM Sleep Induction Area

Corresponding to the peri-LC α in the cat is an area in the rat, which has been termed the sublateralodorsal nucleus (SLD) [75]. The SLD, which has been identified as a REM sleep induction area [76], is in a similar position in the rat to the peri-LC α in the cat and lies ventral to the LC, but caudal to the PnOc (cf. Fig. 3). In the rat, the PnOc does not extend so far in the posterior direction, thus positioning the SLD dorsal to the PnC. Unlike the peri-LC α in the cat and the PnOc in the rat and cat, cholinergic agonists do not induce REM sleep when locally injected into the SLD in the rat [76]. This result, however, does not negate the evidence supporting this area as a REM sleep induction area, since the SLD is one of the few loci in the unanesthetized intact rat that are known to support a short latency REM sleep onset following local injection of pharmacological agents [76, 77]. The SLD should not be confused with a region lying just dorsal and immediately below the LC that has been referred to as the subcoeruleus (SubC) (cf. Fig. 3). The SubC is associated with the generation of P-waves in the rat, the homolog of PGO waves in the cat [78]. The SubC is cholinceptive, and local injection of cholinergic agonists releases the P-waves from their association with REM sleep but does not alter the time spent in REM sleep [78].

The SLD contains REM-on neurons that discharge selectively during REM sleep, and iontophoretic application of the glutamate receptor agonist, kainate, in the SLD of head-restrained rats triggers a REM sleep-like state within minutes of the start of administration [76]. These findings are consistent with the concept that excitation of REM-on neurons in the SLD is sufficient to initiate REM sleep [79–81]. This is further supported by the widespread projections of SLD neurons, both rostrally and caudally, which could be responsible for recruiting various neuronal populations subserving the signs of REM sleep [76, 79]. These targets of SLD influence include bulbar sites implicated in the muscle atonia of REM sleep [76, 82] and direct projections to the ventral horn of the spinal cord [80]. A significant number of fibers from the SLD also innervate the medial thalamus and are observed in the horizontal limb of the diagonal band of Broca, sites implicated in cortical activation [76]. Thus, the SLD is capable of directly influencing at least two of the major signs of REM sleep.

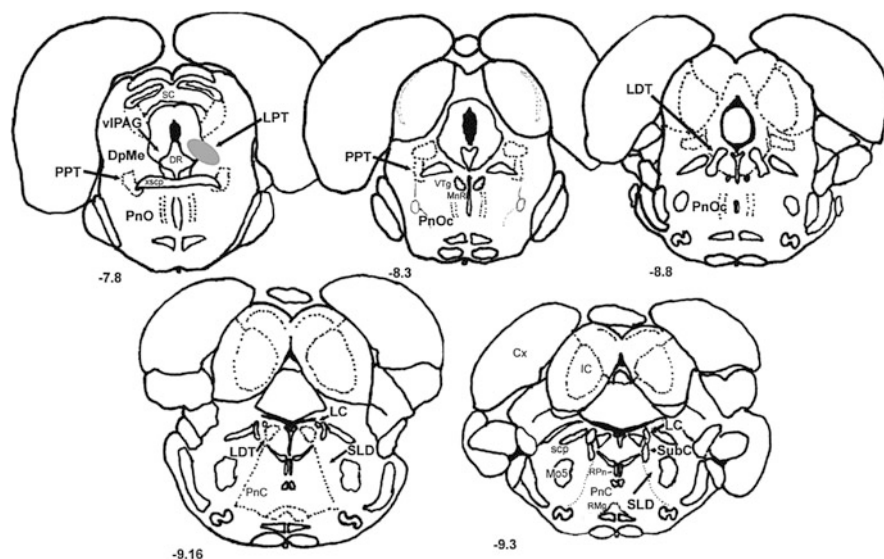


Fig. 3 Schematic drawings of coronal sections through the pons of the rat to illustrate the regions that have been identified as important for REM sleep induction in this species. The region defined as the LPT [80], delineated by the *gray ellipse*, encompasses the viPAG and extends into the DpMe, part of the MRF. The PnOc is a REM sleep induction area lying caudal to the decussation of the scp and rostral to the PnC. More caudal to the PnOc is another REM sleep induction area, the SLD. Dorsal to the SLD is the SubC, a putative P-wave generator site. See text for more details. Indicated below each section is the distance (in millimeters) of the coronal plane of the section caudal to bregma. *Cx* neocortex; *DpMe* deep mesencephalic nucleus; *DR* dorsal raphe nucleus; *IC* inferior colliculus; *LC* locus coeruleus; *LDT* laterodorsal tegmental nucleus; *LPT* lateral pontine tegmentum; *ml* medial lemniscus; *Mo5* motor trigeminal nucleus; *MnR* median raphe nucleus; *PnC* caudal pontine reticular nucleus; *PnO* oral pontine reticular nucleus; *PnOc* caudal part of PnO; *PPT* pedunculopontine tegmental nucleus; *RMg* raphe magnus nucleus; *RPn* raphe pontis nucleus; *SC* superior colliculus; *scp* superior cerebellar peduncle; *SLD* sublateralodorsal tegmental nucleus; *SubC* subcoeruleus; *VTg* ventral tegmental nucleus; *xscp* decussation of the scp

5.5 GABAergic Mechanisms in the SLD

GABA_A antagonists injected locally in the SLD of the rat induce REM sleep, as in the cat peri-LC α . However, cholinergic agonists do not induce REM sleep in the SLD of the rat [76]. Hence, the local mechanism of GABA_A antagonist-induced REM sleep must differ significantly from that in the PnOc. The GABA_A antagonists, BIC and GBZ, either iontophoretically applied in the SLD of head-restrained rats [76], or microinjected into the same region in freely moving rats [77], induce REM sleep within minutes. This induction can occur directly from wakefulness and results in unusually long episodes of REM sleep. Furthermore, the REM sleep-inducing effects of a BIC microinjection typically last less than 60 min [77].

This duration of action is consistent with the time course of diffusion of the compound away from the site, indicating that the induction mechanism requires receptor occupancy. It has also been reported that rats can be maintained continuously in a REM sleep-like state for as long as the BIC ejection continues in the SLD [76]. This is another difference from the REM sleep induction that occurs in the PnOc. In the latter area, REM sleep induction is prolonged for several hours following a single injection [70], which suggests the initiation of a process that continues after the drug has diffused away from the area.

By definition and observation [76], REM-on neurons in the SLD discharge only during REM sleep. Application of kainate, a glutamate receptor agonist, both evokes activity in SLD REM-on neurons and induces REM sleep [76], as does the local application of GABA_A antagonists [76, 77]. These findings are consistent with the hypothesis that activity in the SLD REM-on neurons is sufficient to induce REM sleep [76, 79, 80]. Furthermore, the REM sleep-inducing effect of GABA_A antagonists in the SLD can be blocked by local application of kynurate, a nonselective glutamate antagonist [76]. This suggests that the action of GABA_A antagonists in the induction of REM sleep is disinhibition of a glutamate excitation. The prevailing theory thus posits the tonic presence of both a glutamatergic excitation and a GABAergic inhibition of SLD REM-on neurons, with the REM-on pattern of discharge in these neurons produced by the removal of the GABAergic inhibition only during REM sleep. Application of sufficient kainate can overcome this inhibition and GABA_A antagonists also can remove it, so permitting the REM-on neurons to become active and induce REM sleep. This negative control by GABA in the SLD is currently viewed as one of the critical mechanisms in the distributed system of REM sleep control [76, 79–81, 83].

The specific mechanism by which GABAergic and glutamatergic afferents to the SLD interact is not known. One mechanism could be through a postsynaptic integration of these inhibitory and excitatory inputs. Another possible mechanism could be one that parallels the REM sleep-inducing action of GABA in the PnOc, i.e., GABA_A receptors could act to inhibit glutamate release presynaptically. This question remains to be explored.

5.6 The Source of GABAergic Innervation to the SLD

The SLD receives GABAergic innervation from many areas including local GABAergic neurons intrinsic to the SLD [80, 83, 84]. One criterion for identifying the source of disinhibition is a selective decrease in activity of those neurons during REM sleep. One method that has been used to identify state-related neuronal activation is the expression of the immediate early gene protein product c-Fos. Neuronal activation during a 2–3 h period prior to sacrifice is associated with c-Fos expression [85, 86]. This immunohistological method is used in conjunction with behavioral manipulations that result in groups of animals having either very low amounts of REM sleep or very high amounts of REM sleep in the last few hours

before sacrifice [83, 84, 87, 88]. For example, this can be achieved by subjecting one group to a REM sleep deprivation procedure prior to sacrifice, while the second group is also deprived of REM sleep but allowed a period of recovery so inducing a rebound in REM sleep prior to sacrifice.

Local GABAergic neurons in the SLD are unlikely candidates for the source of inhibition that gates REM sleep. One study found a small, but significant increase in the number of c-Fos-expressing GABAergic interneurons in the SLD in the high REM sleep condition when compared with the low REM sleep condition [87]. But another study found no difference between conditions [83]. Taken together, these results indicate that the local neurons do not have the required pattern of activity, i.e., a lack of activation during REM sleep. An extensive examination of the c-Fos expression patterns in neuronal populations of GABAergic neurons that innervate the SLD found few potential candidate populations that had reduced c-Fos expression in the high REM sleep condition [83]. One potential group comprises those GABAergic neurons that are retrogradely labeled from the SLD in the region of the caudolateral and ventrolateral periaqueductal gray, extending into the deep mesencephalic nucleus, part of the MRF [80, 83, 84]. This region has been termed the lateral pontine tegmentum (LPT) by Lu and colleagues (cf. the gray ellipse in Fig. 3) [80]. Patches of the LPT contain a greater number of GABAergic neurons expressing c-Fos in the low REM sleep condition when compared to the high REM sleep condition [83], indicating, as required for this critical group, a lack of activation during REM sleep. Additional support for a role for the LPT came from a study in which bilateral lesions of the region resulted in a prolonged increase in REM sleep [80]. In addition, as now demonstrated in the cat, rat, and guinea pig, microinjections in the LPT of the GABA_A agonist muscimol, which presumably inhibits almost all neuronal activity in this region, also induced a significant increase in REM sleep [48, 83, 89]. In summary, removing the GABAergic influence of neurons in the LPT increases REM sleep.

Thus, GABAergic neurons in the LPT could provide the disinhibition of REM-on neurons in the SLD. What has not yet been demonstrated, however, is that the GABAergic innervation from the LPT originates from the specific neurons that are not activated during REM sleep and that these neurons actually project to the REM-on neurons in the SLD. To further emphasize this point, a recent study reported that no difference in phosphorylated cAMP response element-binding protein (pCREB), another marker of neuronal activation, was observed in the LPT of rats under conditions of high and low REM sleep [90]. However, the GABAergic neuronal subpopulation or its projection to the SLD was not specifically identified in this study.

GABAergic afferents to the SLD also originate in the other REM sleep induction area, the PnOc, though these are less numerous than from the LPT [84]. Hence, one mechanism by which compounds injected into the PnOc could induce REM sleep would be by inhibiting the GABAergic neurons projecting to the SLD. In vitro recording of identified GABAergic neurons in the PnOc of the mouse indicated that about a third of these neurons were inhibited by carbachol [65]. Although the terminal projections of these GABAergic neurons were not determined in this

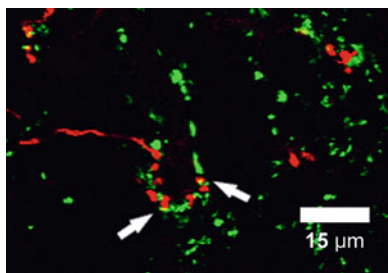


Fig. 4 Fluorescence laser scanning confocal photomicrograph in the SLD of the rat. The ortho-grade tracer, biotinylated dextran amine (*red*) was injected into the PnOc. GABAergic presynaptic terminals are labeled by the vesicular GABA transporter (*green*). Shown is an axon fiber originating from a neuron in the PnOc making presumptive axo-somatic synaptic contacts with a neuron in the SLD. In this view, double-labeled (*yellow*) varicosities, marked by the *arrows*, are evident, indicating the GABAergic nature of these terminals. *Bar* indicates scale

study, carbachol microinjected directly into the PnOc, as noted above, can induce REM sleep, making the PnOc a possible source of the GABAergic influence to the SLD. Inasmuch as ACh levels are highest in the PnOc during REM sleep [29], it can be predicted that these GABAergic neurons would indeed be inhibited during REM sleep. In fact, one study reported that c-Fos expression in GABAergic neurons in the PnO is significantly reduced in the high REM sleep condition when compared with the low REM sleep condition [88]. This is consistent with a lack of activation during REM sleep and, potentially therefore, an inhibition. However, a recent study failed to replicate this finding and reported no significant difference in c-Fos expression between conditions [83]. Presumably, not all GABAergic neurons in the PnOc project to the SLD. Of those that do, we have observed multiple potential axosomatic synapses onto SLD neurons (Fig. 4). This type of synaptic arrangement could have a high degree of inhibitory efficacy. What now remains to be determined is the pattern of c-Fos expression in the GABAergic neurons of the PnOc that specifically project to the SLD, and whether they innervate REM-on neurons.

5.7 *SLD Neurons are Effective for Muscle Atonia*

A major descending projection from the SLD is to the ventral horn of the spinal cord [79]. Most of these SLD neurons are glutamatergic and hypothesized to be involved in the production of muscle atonia in REM sleep [80]. In the rat, there may also be a lesser projection to the ventromedial medulla, which is a major relay for motor inhibition in the cat [82]. Recent preliminary evidence utilizing c-Fos expression has indicated that neurons in the SLD, activated during a period of high REM sleep, are also glutamatergic [91]. These glutamatergic neurons may constitute the population of REM-on neurons hypothesized to be sufficient for REM sleep induction. Alternatively, they may comprise the population of atonia-on

neurons involved specifically in muscle inhibition during REM sleep. Currently, the latter hypothesis has more support, and a likely role for SLD neurons is that they are effective for REM sleep signs. This is indicated by the critical finding that REM sleep can occur without their presence, since bilateral lesions that include the SLD eliminate muscle atonia but not the state of REM sleep [92].

6 Perspectives

It is apparent that our current understanding of the mechanisms of REM sleep is limited. Although advances have been made in identifying some brain regions involved in REM sleep, it is likely that other areas remain to be discovered. Furthermore, the mechanisms by which a particular region might accomplish the control of REM sleep remain, to a large extent, unknown. For example, we have reviewed those brain regions that are sufficient to affect REM sleep expression and yet none has been identified as necessary for the appearance of the state. This is well exemplified by the PnOc. Like other areas in the PRF, the PnOc consists of a heterogeneous collection of neuronal types, and this cellular heterogeneity extends to neurotransmitter utilization, receptor expression, intrinsic membrane properties, and efferent and afferent projections. Hence, difficulty has been encountered in determining which neurons in the PnOc promote REM sleep, which inhibit the appearance of the state and, indeed, which play no role in its expression. In the SLD, however, recent techniques have yielded significant data that have identified individual neurons, their state-related activity, their connectivity to other neurons, and the valence of their interactions. The overall result of these studies is that the SLD appears to be an effective area for the expression of the signs of REM sleep. The fact that the SLD is also identified as a REM sleep induction area is therefore critical, because it suggests that induction of some of the signs of REM sleep may be sufficient to induce the cascade of events that leads to the complete expression of the state.

By extension, the results obtained in the SLD also show the importance of connectivity and interactions in the mechanisms of REM sleep. Conceptually, determining the state-related pattern of neuronal discharge and connectivity would appear essential to elucidating such a distributed and interactive system. However, interpretation of such data may not be obvious. For example, we have reviewed the large number of neuronal inputs that can modulate ACh release at cholinergic terminals in the PnOc. The conclusion is that the release of ACh in the PnOc can be decoupled from the generation of action potentials in cholinergic neurons projecting from the LDT/PPT. Similarly, the activity of neurotransmitter transporters can determine the duration of neurotransmitter action in the synaptic cleft as well as potential diffusion to peri-synaptic receptors. Under certain conditions, transporters can reverse their activity, resulting in neurotransmitter release that is independent of action potentials. GABA transporters can thus influence extracellular concentrations of GABA that activate extrasynaptic

receptors. In summary, it is evident that understanding this complexity will require continued exploration of the many types of mechanisms that underlie neuronal interactions.

In addition to neurotransmitter release, the effective functional expression of postsynaptic receptors determines neuronal communication. As we have noted, antagonism at GABA_A receptors in the pontine REM sleep induction areas induces the state, and hence a similar result could follow if the relevant receptors were downregulated. Trafficking of GABA_A receptors in and out of the membrane is a rapid process, capable of dynamic regulation on the time scale of inter-REM sleep intervals. Such a mechanism might also be implicated in the processes that regulate the state over prolonged intervals, such as the REM sleep rebound that follows sleep deprivation. In addition, after activation of the GABA_A receptor and opening of the chloride channel, transmembrane chloride gradients control the degree and valence of membrane polarization. An ion transporter, such as the potassium/chloride cotransporter (KCC2), which establishes these gradients in neurons, is also dynamically regulated. Hence its regulation, like the downregulation of the GABA_A receptor, might be an important REM sleep mechanism. Subsequent to receptor activation by most neurotransmitters, cascades of intracellular events, including gene transcription and protein–protein interactions, follow. Any of these processes could have crucial consequences for the integrative functions of individual neurons and the interactions among them. The molecular neurobiology of changes in vigilance state is only now being explored for the first time. For example, the prolonged inhibition of cAMP production in PnOc neurons during the expression of REM sleep may be an indication of a long-lasting neural adaptation that subserves state control.

Finally, we should note that neuronal communication can occur in ways that are unrelated to synaptic transmission. Gap junctions between neurons in the SLD have been detected. This form of electrical communication can affect the network properties and so influence the expression of state-related patterns of activity in individual neurons. The interaction of neurons with glial cells in the REM sleep induction areas and, indeed, throughout the brain, also remains to be investigated.

7 Conclusion

We have limited our discussion in this review to pontine GABAergic and cholinergic interactions and their role in REM sleep mechanisms. Additional neurotransmitter systems have been briefly mentioned and more are discussed in detail in the other chapters of this volume. But neurotransmitter systems are only one level of organization of the brain. Research into the integration of many different types of systems over a range of levels of organization is now required. The challenge is, indeed, one of elucidating the principals of the operation of complex nervous systems. Our hope here is that we have provided clues to future areas of research

that will eventually help develop an understanding of the neural mechanisms of REM sleep generation and control.

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Melatonin and Sleep: Possible Involvement of GABAergic Mechanisms

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Abstract Pineal melatonin is synthesized and secreted in close association with the light/dark cycle. The temporal relationship between the nocturnal rise in melatonin secretion and the “opening of the sleep gate” (i.e., the increase in sleep propensity at the beginning of the night), coupled with the sleep-promoting effects of exogenous melatonin, support the view that endogenous melatonin is involved in the regulation of sleep. The sleep-promoting and sleep–wake rhythm-regulating effects of melatonin are attributed to its action on MT₁ and MT₂ melatonin receptors present in the suprachiasmatic nucleus (SCN) of the hypothalamus. Nearly all neurons in the SCN contain γ -aminobutyric acid (GABA), and GABA-driven mechanisms have been shown to play a key role in circadian coupling among regions and neurons in the SCN. Results are reviewed indicating that among the neurochemical effects of melatonin in the SCN, its interaction with the GABAergic system appears to be significant for coupling to sleep mechanisms.

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1 Introduction

Melatonin, the hormone synthesized and secreted by the pineal gland, is secreted during darkness in all mammals. Production of melatonin is controlled by the master circadian clock located in the suprachiasmatic nucleus (SCN). Melatonin plays a central role as a chemical messenger from the body clock that, acting at both central and peripheral sites, conveys the signal of darkness to the organism.

In humans, melatonin administration has two major effects on the sleep–wake cycle: it influences the timing of the sleep–wake rhythm and produces drowsiness. Melatonin's effect on the timing of rhythms is due to feedback effects on the SCN while its effects on sleepiness may be due to actions at other central sites also.

γ -Aminobutyric acid (GABA) has long been recognized as an important neurotransmitter in the circadian apparatus. Nearly all neurons in the SCN contain GABA [1], and GABAergic mechanisms have been shown to play a key role in coupling among regions [2] and neurons [3, 4] within the SCN.

Among the neurochemical effects of melatonin, its interaction with the GABAergic system is thought to be significant [5]. GABA_A receptor currents are modulated by melatonin in neurons of the rat SCN and hippocampus [6], chick spinal cord [7], and carp retina [8]. The purpose of this chapter is to discuss the available evidence that indicates that GABA-containing neurons can be instrumental in the circadian and sleep-promoting effects of melatonin.

2 Regulation of Melatonin Production

Although melatonin is synthesized in a wide variety of tissues [9, 10], circulating melatonin comes almost entirely from the pineal gland. Its synthesis in the pineal is regulated by the master clock in the SCN; under certain circumstances, such as exposure to light at night, this clock signal can be overridden downstream from the clock by visual inputs, which suppress melatonin synthesis.

In mammals, melatonin is synthesized in the pineal gland in a rhythmic manner with high levels during nighttime and low levels during daytime [11–13]. The photoperiod is the predominant influence on melatonin synthesis, entraining the rhythm-generating system in the SCN via the retinohypothalamic tract (RHT) and the geniculohypothalamic tract (GHT). The RHT originates from a subset of retinal ganglion cells that contain the photopigment melanopsin and the neurotransmitters pituitary adenylyl cyclase-activating peptide (PACAP) and glutamate [14, 15]. Retinal projections to the intergeniculate leaflet of the lateral geniculate complex in turn project to the SCN via the GHT [16, 17]. Light acts via these tracts to entrain the rhythm in the SCN as well as acting downstream of the SCN clock to block activity of the pineal gland.

Projections of the SCN driving the daily melatonin rhythm inhibit the firing of neurons in the subparaventricular zone [18]. From this zone, a hardwired

multisynaptic pathway starts, which includes the medial forebrain bundle, reticular formation and intermediolateral cell column of the cervical spinal cord, the superior cervical ganglion, and postganglionic sympathetic fibers that end in the vicinity of pineal cells and that stimulate melatonin synthesis [19–21]. It was proposed that the SCN generates the daily rhythm in plasma melatonin concentrations via the combination of a continuous (glutamatergic) stimulation of the preautonomic neurons in the paraventricular nucleus (PVN) and a nocturnal withdrawal of the inhibitory (GABAergic) SCN inputs to these neurons [22]. A somatotopically organized GABAergic SCN output inhibits selective groups of PVN preautonomic neurons at specific times of the light/dark cycle.

Norepinephrine (NE) released from the pineal sympathetic nerve endings acts on pinealocytes to stimulate melatonin synthesis via a dual receptor mechanism. NE activates adenylyl cyclase by acting on β_1 -adrenergic receptors [23] and protein kinase C activity by acting on α_{1B} -adrenergic receptors [24]; the latter effect potentiates β_1 -adrenergic receptor activation of adenylyl cyclase. This receptor cross-talk causes a rapid, large increase in cyclic 3',5'-adenosine monophosphate (cAMP), which leads to phosphorylation of the enzyme arylalkylamine *N*-acetyltransferase (AANAT). When phosphorylated, AANAT becomes activated by formation of a reversible regulatory complex with 14-3-3 proteins [25].

AANAT, which converts serotonin to *N*-acetylserotonin, has a pivotal role in the timing of melatonin synthesis [25]. It increases very rapidly with a doubling time of about 15 min in response to darkness onset and it decreases in response to light at an even more rapid half life of degradation (3.5 min). Since melatonin itself has an overall half life in the circulation of about 20–30 min in man, its levels change promptly in response to circadian signals and light [26, 27].

Hydroxyindole-*O*-methyl transferase (HIOMT), which catalyzes production of melatonin from *N*-acetylserotonin, may well be responsible for the amplitude of the nocturnal peak of melatonin [28, 29]. Studies in rats using a combination of molecular probes together with a sensitive *in vivo* measurement of pineal indoles have indicated that *N*-acetylserotonin is present in vast excess during the night [30]. Based on these and other findings, it was concluded that although AANAT is the rhythm-generating enzyme, it is not rate-limiting for nocturnal production, which is controlled by HIOMT. HIOMT activity in the rat is regulated both by a short-term nonadrenergic stimulus [31] and by a longer term adrenergic stimulus [31, 32]. Melatonin synthesis in the pineal gland is also influenced by neuropeptides, such as vasoactive intestinal peptide, PACAP, and neuropeptide Y, which are partially coreleased and seem to potentiate the NE response [33]. Other receptors, e.g., for steroid hormones, are present in the pineal gland and may underlie the correlation between the melatonin rhythm and that of reproductive hormones [34–38].

Melatonin production exhibits considerable interindividual differences. Some subjects produce more melatonin during their lifetime than others, but the significance of this variation is not known. Studies of twins suggest that these differences may have a genetic basis [39].

3 Melatonin Metabolism

Since melatonin is a lipophilic substance, once it is synthesized in the pineal gland, it diffuses readily into the blood stream. In the circulation, melatonin is partially bound to albumin [40] and can also bind to hemoglobin [41]. Melatonin rapidly disappears from the blood with a half-life of about 20–30 min, depending on the species examined. Melatonin half-life is biexponential, with a first distribution half-life of 2 min and a second of 20 min [42]. Melatonin released to the CSF via the pineal recess attains concentrations in the third ventricle that are up to 20–30 times higher than in the blood [43]. These concentrations, however, rapidly diminish with increasing distance from the pineal, suggesting that melatonin is taken up by brain tissue [44].

Circulating melatonin is metabolized mainly in the liver, which clears 92–97% of circulating melatonin in a single pass [45]. Melatonin is first hydroxylated in the C6-position by cytochrome P₄₅₀ monooxygenases (isoenzymes CYP1A2, CYP1A1, and to a lesser extent CYP1B1) and thereafter conjugated with sulfate to be excreted as 6-sulphatoxymelatonin, glucuronide conjugation being extremely limited [42]. CYP2C19 and, at lower rates, CYP1A2 also demethylate melatonin to its precursor *N*-acetylserotonin [46].

The metabolism in extrahepatic tissues exhibits substantial differences. Tissues of neural origin, including pineal gland and retina, contain melatonin-deacetylating enzymes, which are either specific melatonin deacetylases [47] or less-specific aryl acylamidases; since eserine-sensitive acetylcholinesterase has an aryl acylamidase side activity, melatonin can be deacetylated to 5-methoxytryptamine in any tissue carrying this enzyme [47]. Therefore, melatonin metabolism in the brain involves oxidative pyrrole-ring cleavage. No 6-hydroxymelatonin is detected after melatonin injection into the cisterna magna [48]. This pathway may be particularly important because, as above mentioned, melatonin is also released via the pineal recess into the cerebrospinal fluid as well as into the circulation [43]. The primary cleavage product is *N*¹-acetyl-*N*²-formyl-5-methoxykynuramine (AFMK), which is deformylated, either by arylamine formamidase or hemoperoxidases, to *N*¹-acetyl-5-methoxykynuramine (AMK). Surprisingly, numerous enzymatic (indoleamine 2,3-dioxygenase, myeloperoxidase), pseudoenzymatic (oxoferryl hemoglobin, hemin), photocatalytic, or free-radical reactions lead to the same product, AFMK [49]. Some estimations indicate that pyrrole ring cleavage contributes to about one third of the total catabolism of melatonin, but the percentage may be even higher in certain tissues. Other oxidative catabolites are cyclic 3-hydroxymelatonin, which can also be metabolized to AFMK, and a 2-hydroxylated analog, which does not cyclize but turns into an indolinone [49]. Additional hydroxylated or nitrosated metabolites have been detected, but they appear to be present in minor quantities only. AFMK and AMK also form metabolites by interactions with reactive oxygen and nitrogen species. AFMK is produced by numerous nonenzymatic and enzymatic mechanisms [49]; its formation by myeloperoxidase appears to be important in quantitative terms [50]. Antioxidative protection, safeguarding of mitochondrial

electron flux and, in particular, neuroprotection have been demonstrated in many experimental systems to be mediated by melatonin and its endogenous metabolites.

4 Melatonin Receptors

4.1 Identification and Regulation

Binding sites for melatonin were initially identified in a wide variety of central and peripheral tissues using ^3H -melatonin [51, 52] and later 2- I^{125} -iodomelatonin [53]. Molecular cloning of the first high affinity melatonin receptor (MT_1) by Reppert and coworkers was accomplished using a cDNA library constructed from a dermal cell line of melanophores, the first tissue in which melatonin's action had been demonstrated [54]. This initial finding led to the discovery that there are at least two G_i -protein-coupled melatonin receptors in humans. The second receptor (MT_2) [8] is 60% identical in amino acid sequence to the MT_1 receptor. Yet a third receptor, now called GPR50, shares 45% of the amino acid sequence with MT_1 and MT_2 but does not bind melatonin [55]. It is unusual in that it lacks N-linked glycosylation sites and has a C-terminal that is over 300 amino acids long.

A fourth 2- I^{125} -iodomelatonin-binding site was identified in mammals [56] (MT_3 , initially called ML-2). Unlike the picomolar affinity membrane receptors, it is a nanomolar affinity-binding site with a different specific pharmacologic profile and fast kinetics of association/ dissociation [57]. It has now been purified from hamster kidney and characterized as quinone reductase type 2 (QR2 or NQO2) [58].

In addition, functional and ligand-binding studies have demonstrated the presence of low affinity (K_D in the 10 nmol range) membrane-binding sites for melatonin in the preoptic area of the hypothalamus and in the medulla-pons in rats [59, 60] and hamsters [61]. While the density of these sites varies with the time of day and physiological conditions, compatible with the ability of melatonin to inhibit dopamine release in these areas [62], the proteins involved in this activity have not been identified so far.

A combination of approaches utilizing molecular clones and pharmacologic tools has revealed a considerable amount of information about the MT_1 and MT_2 receptors. For example, it has been shown that the MT_1 receptor inhibits firing acutely in SCN slices, and that principally MT_2 , but also MT_1 , may contribute to phase shifting in these slices [63]. MT_1 and MT_2 may also regulate differentially GABA_A receptor function in SCN [6].

Recently, it has been shown that many G protein-coupled receptors (GPCR), including the MT_1 and MT_2 receptors, exist in living cells as dimers. The relative propensity to form the MT_1 homodimer or the MT_1/MT_2 heterodimer formation is similar whereas that of the MT_2 homodimer is 3–4-fold lower [64, 65]. It is of considerable interest that the GPR 50 receptor, though lacking the ability to bind melatonin, abolishes high affinity binding of the MT_1 receptor through

heterodimerization [66, 67]. Thus, the GPR50 receptor may have a role in melatonin function by altering binding to the MT₁ receptor.

Mapping of the MT₁ and MT₂ receptors in the brain, though not yet complete, has revealed much information. As expected, MT₁ and MT₂ receptors are present in the SCN [68, 69]. But they are also found in many other brain areas and in the periphery. The MT₁ receptor is widely distributed in the hypothalamus: of particular note, it is colocalized with corticotrophin in the PVN and with oxytocin and vasopressin in the PVN and supraoptic nucleus [70]. MT₂ receptors have been identified in the hippocampus [71], MT₁ and MT₂ in the occipital cortex [72], and MT₁ in the dopaminergic system [73]. Effects produced by alterations in melatonin patterns or levels may be secondary to responses in any of these or other regions yet to be characterized.

Melatonin is also a ligand for a retinoid-related orphan nuclear hormone receptor (RZR/ROR α) [74]. These nuclear receptors belong to the RZR/ROR orphan receptor subfamily, which includes three subtypes (α , β , γ) and four splicing variants of the α -subtype [75]. In addition, melatonin interacts with intracellular proteins such as calmodulin [76] or tubulin [77, 78] and antagonizes the binding of Ca²⁺ to calmodulin [79]. These interactions are most likely related to some of the physiological effects of melatonin, but critical data regarding this point have yet to be obtained. Direct inhibition of the mitochondrial permeability transition pore by melatonin [80] may suggest that a mitochondrial binding site is involved, although at the present time this has not been confirmed.

Regulation of signal transduction events is essential for maintaining timely and efficient cellular responses and homeostasis. Several studies have demonstrated that exposure of receptors to their agonists results in desensitization so that there is a reduced response to subsequent stimulation. This attenuation of subsequent responses to a hormone or drug may be classified as either homologous (agonist-specific) or heterologous (agonist-nonspecific) desensitization, and it may be due to various mechanisms including receptor phosphorylation, sequestration and down-regulation [81]. A diurnal rhythm in the density of high-affinity receptors for melatonin, which is inversely related to its circulating levels, was observed in hamster and rat brain membranes [82] and in rat basal hypothalamic membranes containing the SCN [83]. Thus, high-affinity binding was highest late in the light phase, following prolonged depletion of the endogenous agonist, and lowest during darkness when exposure to elevated melatonin concentrations presumably down-regulated the high-affinity receptor [82, 83]. Similarly, a significant increase in high-affinity binding has been observed in the pars tuberalis/median eminence of rats killed at the end of the light phase (when melatonin levels are low) as compared with animals killed in the morning [84].

Suppression or depletion of circulating melatonin levels by exposure to constant light or pinealectomy caused a significant increase in the density of high-affinity sites in rat brain membranes [85] as well as in the pars tuberalis of the rat and hamster [86]. Conversely, a single injection of melatonin reversed the effect of constant light or pinealectomy on high-affinity binding in the rat pars tuberalis and SCN [87]. Moreover, preincubation of cultured ovine PT cells in the presence of

melatonin (100 pM or 1 μ M) for 24 h resulted in a significant decrease in melatonin binding in crude pars tuberalis membranes [88].

The MT₁ and MT₂ melatonin receptors are differentially and distinctly regulated by physiological (30–400 pM) and supraphysiological (1–1000 nM) concentrations of melatonin. Physiological concentrations of nocturnal melatonin (100–400 pM) are already well above its affinity (K_D) for the melatonin receptors in their high affinity state, which are activated by low picomolar concentrations of melatonin. Daytime concentrations typically fall below 30 pM, and yet they can induce activation and desensitization of melatonin receptors upon prolonged exposure to the hormone (approximately 8 h) [89, 90]. The blood levels of melatonin following administration of an oral dose of 0.3 mg to humans are similar to endogenous levels found at night [91]. However, oral doses of melatonin or other melatonin receptor agonists at 1 mg or higher may increase blood levels several times above the concentration necessary to activate melatonin receptors and therefore may alter receptor sensitivity [92, 93].

Given the potential involvement of MT₁ and MT₂ receptors in phase shifting circadian rhythms in mammals, persistent desensitization of these receptors by supraphysiological levels of melatonin could affect circadian rhythmicity and sleep [89, 90]. Phase advance of the circadian rhythm of neuronal firing in rat SCN brain slices by melatonin applied at CT 23 (Circadian Time (CT) 12: onset of activity in nocturnal animals) is mediated through activation of MT₂ receptors, which are functionally desensitized by exposure to physiological levels of melatonin (300 pM) for a length of time mimicking the nocturnal surge (8 h). Concurrent exposure of the SCN brain slice to both melatonin and the MT₂ antagonist 4-phenyl-2-propionamidotetraline (4P-PDOT) blocks the functional desensitization of MT₂ receptors [89, 90]. The foregoing and other studies suggest that while both the MT₁ and MT₂ receptors can be desensitized by exposure to melatonin, the receptors are differentially regulated depending on melatonin concentration (physiological vs. supraphysiological), time of exposure (e.g., short vs. long), cellular background, and receptor state (quiescent vs. constitutively active) [89, 90, 94].

Other agents such as neurotransmitters, hormones, or clinically administered drugs may also be involved in the regulation of MT₁ and MT₂ receptors. For example, the potent steroid hormone 17 β -estradiol causes a significant increase in the density of binding sites for 2-[¹²⁵I]-iodomelatonin in Chinese hamster ovary cells transfected with the human MT₂ receptor and attenuates melatonin signaling via the human MT₁ subtype [95]. Another example is given by valproic acid (VPA; 2-propylpentanoic acid), a short-chain branched fatty acid, which is widely used as an anticonvulsant and mood stabilizer. VPA can upregulate the MT₁ receptor in rat C6 glioma cells [96] and is known to increase the levels of GABA via stimulation of GABA biosynthesis and inhibition of enzymes involved in GABA degradation [97]. Although there is evidence for an interaction between melatonin and GABAergic systems with implications for diverse aspects of brain function including sleep regulation [5, 98–100], experiments with the GABAergic antagonist bicuculline do not support a GABAergic mechanism for induction of MT₁ receptors in C6 cells, which may lack functional GABA receptors [96]. Nonetheless, these

findings raise interesting questions about the potential role of melatonin and its receptors in the diverse clinical effects of VPA. For example, it is possible that the anticonvulsant and mood-stabilizing effects of VPA may be enhanced by melatonin [101, 102]. Interestingly, add-on melatonin has been reported to improve sleep behavior in epileptic children treated with VPA [103].

4.2 Receptor Signaling

The melatonin MT_1 and MT_2 receptors mediate inhibition of the adenylyl cyclase-cAMP pathway via pertussis toxin-sensitive G_i proteins. After being first described in frog melanophores [104], melatonin-mediated decreases in cAMP have been observed in a number of mammalian tissues including pituitary, SCN, and cerebral arteries [53, 105].

In addition, the MT_1 receptor mediates the potentiating effect of melatonin on phospholipase C activation and arachidonate release following PGF_{2a} treatment [106]. This effect of melatonin is also sensitive to pertussis toxin, suggesting involvement of an inhibitory G protein. A possible activation of the protein kinase C (PKC) pathway by melatonin via the MT_1 receptor should be also considered [107, 108] (Fig. 1).

MT_1 melatonin receptors can regulate ion fluxes and specific ion channels. One of these channels is the inward rectifier potassium channels (Kir) (Fig. 1).

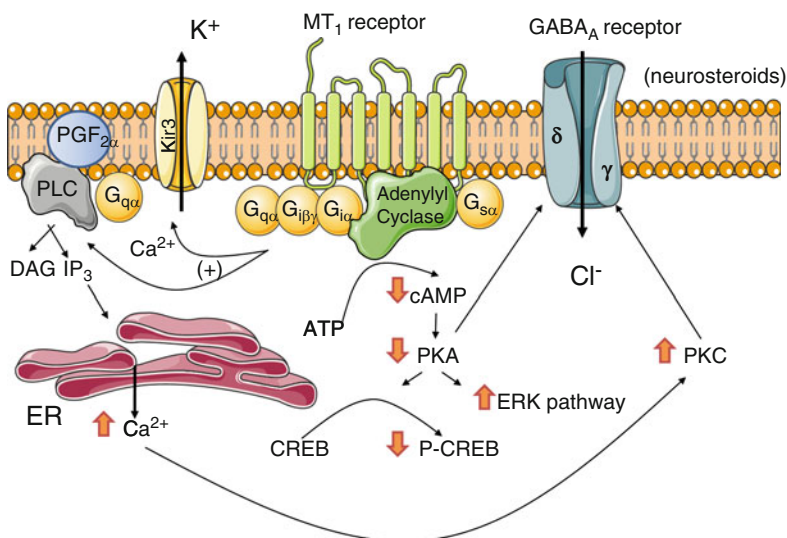


Fig. 1 Possible cellular pathways involved in the physiological modulation of GABAergic function by melatonin via its MT_1 receptor. ER endoplasmic reticulum. See text for further explanation

MT₁ melatonin receptors expressed in *Xenopus* oocytes [109] or AtT20 cells [110] activate Kir3 inward rectifier potassium channels through a pertussis toxin-sensitive mechanism that may involve $\beta\gamma$ subunits of G_i proteins. The activation of Kir3 channels underlies melatonin-induced increases in potassium conductance which, in addition to an enhancement of GABA-ergic activity [6], provides another mechanism by which melatonin inhibits neuronal firing in the SCN [111–113].

Melatonin has been reported to stimulate the mitogen-activated protein kinase-extracellular regulated kinase (ERK) pathway in MT₁-transfected Chinese hamster ovary cells [114] and in the mouse GT1-7 neuronal cell line, which expresses this receptor [115], indicating that the MT₁ receptor subtype is involved. Studies in a model of ischemic stroke suggest that the acute neuroprotective effect of melatonin involves activation of the phosphatidyl inositol-3-kinase/Akt pathway, whereas ERK-1/2 and c-Jun N-terminal kinase-1/2, in addition to Akt signaling, appear to be involved in its long-term effects [116]. Thus, the data available indicate that activation of MT₁ melatonin receptors elicits a variety of tissue-dependent signaling responses (Fig. 1).

Recombinant MT₂ melatonin receptors have been shown to couple to inhibition of cAMP formation [8, 117]. In addition, activation of MT₂ melatonin receptors can lead to inhibition of cGMP formation [117]. In the SCN, melatonin increases PKC activity mainly through activation of MT₂ melatonin receptors, as this response is blocked by the selective MT₂ receptor antagonist 4P-PDOT [118]. In the retina, MT₂ melatonin receptors inhibit neurotransmitter release through a mechanism that likely involves intracellular calcium regulation [119].

Collectively, the results indicate that melatonin can interact with multiple cellular pathways to produce its diverse physiological effects. Moreover, MT₁ and MT₂ receptors can interact with divergent signaling pathways, as shown by their ability to mediate stimulation or inhibition of GABAergic activity in the rat brain, respectively [6], or to couple to multiple G proteins [120]. In keeping with this view, the MT₂, but not the MT₁, receptor subtype is reportedly coupled to the cGMP pathway [121].

4.3 Physiological Involvement of Melatonergic Receptors in SCN Neuronal Function

In the SCN, functional MT₁ and MT₂ receptors have been characterized in pharmacological studies using the two melatonin receptor antagonists, luzindole and 4P-PDOT, as well as in studies of mice with genetic deletion of either the MT₁ and/or MT₂ receptors [106, 119, 122, 123]. Melatonin applied in vitro to rodent SCN brain slices inhibits neuronal firing in a concentration-dependent manner [63, 112]. Melatonin inhibits neuronal firing in SCN brain slices from wild type and MT₂ KO but not MT₁ KO mice [63, 123]. Together, these data suggest that the MT₁ receptor mediates the inhibitory effect of melatonin on neuronal activity in the SCN.

Also in the SCN, melatonin, through activation of MT₁ melatonin receptors, inhibits PACAP-stimulated phosphorylation of the transcription factor CREB, an early event in the signaling cascade leading to phase-shifts of the endogenous clock [122, 123]. These effects of melatonin were not observed in the MT₁ KO mice.

To determine the melatonin receptor type (MT₁ and/or MT₂) involved in phase shifting circadian rhythms in the SCN, circadian rhythms of neuronal firing *in vitro* as well as overt activity rhythms *in vivo* were measured [69]. Melatonin (1–10 pM), given at CT10, phase advances by about 3–4 h the circadian rhythms of neuronal firing in rat and mouse SCN. This effect was blocked by the MT₂-selective melatonin receptor antagonist 4P-PDOT in rat and mouse SCN [118, 124] and was absent in SCN from mice with genetic deletion of the MT₂ receptor (MT₂KO) [69].

Interestingly, it was reported that the phase shift of neuronal firing rhythms induced by the MT₁/MT₂ melatonin agonist 2-iodomelatonin (10 pM) was of smaller magnitude in the SCN brain slice from MT₁ KO mice than in that from the wild type mice, suggesting a potential role for MT₁ melatonin receptor activation [63]. Hence, both the MT₁ and MT₂ receptors may mediate phase advances, with the shift induced through activation of the MT₂ receptor being significantly larger. In the Siberian hamster, which does not encode a functional melatonin MT₂ melatonin receptor, melatonin at 1 nM induced a robust phase shift of the circadian rhythm of neuronal firing rate in the SCN slice [125].

In vivo, melatonin treatment did phase shift circadian rhythms of wheel running activity in the wild type and MT₂KO mice kept in constant dark when given 2 h before onset of activity, but not in the MT₁KO or MT₁/MT₂KO, suggesting that *in vivo* activation of the MT₁ receptors phase advances overt circadian rhythms [69]. The paradoxical findings (i.e., phase shift by MT₂ activation in the SCN *in vitro* and by MT₁ activation *in vivo*) are difficult to explain at the present time.

5 Role of SCN in the Regulation of Sleep

The daily sleep/wake cycle is influenced by two separate processes (1) the endogenous biological clock that drives the circadian rhythm of sleep/wake cycle (Process C, for “circadian”) and (2) a homeostatic component (Process S, for “sleep”) that influences sleep propensity, a state that is determined by the immediate history of sleep and wakefulness and additionally by the duration of previous sleep episodes [126]. These two processes, which interact continuously, determine the consolidated bout of sleep at night and the consolidated bout of wakefulness during daytime.

A “forced desynchrony paradigm” (in which the subject’s circadian rhythms are experimentally desynchronized) has been used to study the contribution of each of these components. Observations of subjects whose circadian rhythms had been experimentally desynchronized have supported the inference that rapid eye movement (REM) sleep is driven by the circadian component whereas slow wave (non-REM) sleep is driven by the homeostatic component [127].

The circadian system is also known as a wake-promoting system because it determines the strength of wakefulness [126]. The circadian rhythm in the secretion of the pineal hormone melatonin has been shown to be responsible for the sleep rhythm in both normal and blind subjects (i.e., in the absence of the synchronizing effect of light). It may be possible that melatonin feeds back on the SCN to inhibit the circadian signal responsible for promoting wakefulness [128–130].

Since the SCN interacts with both sleep regulatory mechanisms, Process S and Process C, it was suggested that a functional disruption of the SCN is involved in the disorders of sleep and wakefulness [131]. The role of the SCN in sleep regulation was first studied in the squirrel monkey. In this primate species, lesions made in the SCN caused either loss of sleep or prolonged sleep [132]. It is suggested that circadian signals emanating from the SCN promote wakefulness during the day and may facilitate sleep during the subjective night. It is interesting to note that the neural pathways from the SCN that promote wakefulness are complemented by those that are involved in the promotion of sleep [131].

The molecular machinery that drives circadian rhythmicity occurs in individual SCN neurons [133]. This is not to imply that all SCN neurons are the same; in fact, a wide range of evidence is emerging for functionally distinct cell populations within the SCN [134, 135]. Anatomical evidence supports the broad division of the SCN into distinct ventral (core) and dorsal (shell) subdivisions [136]. Glutamic acid decarboxylase (GAD), the enzyme responsible for synthesizing GABA, is found in nearly all neurons of the SCN [17], and both GABA_A and GABA_B receptors are expressed in this structure [137]. SCN neurons receive a tonic GABA_A receptor-mediated synaptic input that, at least partly, originates within the SCN itself and peaks during the night [138].

Functionally, the exogenous application of GABA can synchronize the electrical activity of SCN neurons [3]; however, GABAergic signaling does not appear to be required for cultured SCN neurons to remain synchronized [4]. There is evidence to suggest that GABA-mediated mechanisms are responsible for coupling ventral and dorsal cell populations in the SCN [2].

A variety of GABA receptor subunits are expressed in the SCN, and GABA receptors are seen in pre, post and extrasynaptic locations [139]. Pharmacological studies have provided evidence for the presence of both γ - and δ -subunit-containing GABA_A receptors in the SCN [140, 141]. Injections of the GABA_A agonist muscimol into the SCN *in vivo* inhibits photic phase shifts at night and blocks light-induced increases in *c-fos* immunoreactivity and Period 1 and 2 gene expression in the SCN. Conversely, muscimol induces daytime phase advances when applied to the SCN either *in vivo* or *in vitro* and decreases daytime Period 1 expression in the SCN [142]. Gaboxadol, a GABA agonist that selectively activates GABA_{A δ} receptors, was shown to inhibit light-induced phase shifts in hamsters *in vivo* but did not induce daytime phase shifts on its own [141]. Therefore, there is a clear difference in the effects on the SCN circadian clock of activating GABA_{A γ} receptors, which generate phasic inhibition, vs. GABA_{A δ} receptors, which tonically inhibit neuronal activity.

5.1 Putative Neuropharmacological Mechanisms Involved in Sleep Modulation by Melatonin

Following the demonstration that daily administration of melatonin to the rat would synchronize the circadian rhythms [143] and that this effect was abolished in animals with SCN lesions, human studies confirmed that melatonin can synchronize the sleep cycle [12]. Moreover, it was shown that blind individuals with desynchronized sleep–wake cycles could be successfully treated with melatonin [144, 145] and that even sighted individuals with a desynchronized rhythm could be treated successfully [146]. These effects point to the SCN as a target for synchronizing actions of melatonin.

The fact that the nocturnal increase of melatonin secretion occurs approximately 2 h in advance of the individual's habitual bedtime [147, 148] has prompted many investigators to suggest that melatonin is involved in the physiological regulation of sleep [149]. The period of wakefulness immediately prior to the increase in sleep propensity is known as the “forbidden zone” for sleep [150, 151]. During this period, sleep propensity is at its lowest level and SCN neuronal activity is very high [152, 153] because melatonin levels are very low. As soon as melatonin levels increase, the “opening of the sleep gate” takes place and sleep propensity augments sharply [152–154]. The secretion of melatonin, which may have both sleep-promoting and circadian rhythm-regulating activities, peaks during the period of greatest increases in sleep propensity [154, 155].

Although the physiological mechanisms underlying modulation of sleep by melatonin await clarification, an interaction with GABAergic systems seems to be warranted. There is *in vivo* electrophysiological evidence that nanomolar concentrations of melatonin can potentiate GABAergic inhibition of neuronal activity in the mammalian cortex [156]. More recently, *in vitro* studies have indicated that the MT₁ receptor is coupled to the stimulation of GABAergic activity in the hypothalamus, whereas the MT₂ receptor mediates an opposite effect in the hippocampus [6].

GABA_A receptor currents are modulated by melatonin in neurons in the rat suprachiasmatic nucleus and hippocampus [6], chick spinal cord [7], and carp retina [8]. When the effect of GABA and melatonin on GnRH neurons isolated from GnRH-EGFP transgenic rats was examined by patch-clamp, it was shown that GABA activated GABA_A receptor currents in the cell body of rat GnRH neurons, and that this effect was modulated by melatonin in a sex-specific manner [157].

Several studies have demonstrated that large doses of melatonin, which greatly exceed the endogenous levels of this hormone, produce sedative/hypnotic effects [158] and can alleviate the symptoms of jet lag in humans [159, 160]. At these pharmacological doses, melatonin could directly interact with benzodiazepine (BZP) receptors, which are allosterically linked to modulation of GABAergic activity via the BZP-GABA_A receptor complex [99, 161]. For example, melatonin competes for [³H]diazepam binding sites in rat, human, and bovine brain

membranes with micromolar affinity [162]. Similarly, pharmacological doses of melatonin act on BZP-GABA_A receptors to enhance both in vitro and in vivo binding of GABA, and to allosterically inhibit binding of the caged convulsant, *t*-butylbicyclopophosphorothionate (TBPS), on GABA-gated chloride channels in rat brain [161].

Pharmacological studies have shown that the anxiolytic, anticonvulsant, and other psychotropic actions of melatonin, which are similar to those exhibited by BZPs, involve the enhancement of GABAergic activity [5, 163–165]. It is thought that GABA binds to specific sites at the interface of the α and β subunits on the BZP/GABA_A receptor complex to induce opening of chloride channels, with consequent membrane hyperpolarization and neuronal inhibition [166]. Central-type BZP, such as clonazepam, bind to other sites on the BZP/GABA_A receptor complex to allosterically enhance GABAergic activity. The binding site for melatonin on the BZP-GABA_A receptor complex is not known, but its ability to competitively inhibit [³H]diazepam binding suggests a direct interaction within the BZP binding pocket, which is located at the α/γ subunit interface of the BZ-GABA_A receptor complex [166, 167]. In view of the importance of GABAergic mechanisms in sleep modulation, it is likely that the sedative effects of pharmacological doses of melatonin involve its allosteric interaction with BZP-GABA_A receptors. This view is supported by evidence that BZP-GABA_A antagonists block the sleep-inducing effect of pharmacological doses of melatonin in experimental animals [168].

In addition to its interaction with central-type BZP receptors, pharmacological concentrations of melatonin can also bind to peripheral-type BZP receptors, which are involved in neurosteroidogenesis [169]. Neurosteroids, which appear to be primarily produced by astrocytes [170], can exert potent modulatory effects on ion-gated neurotransmitter receptors including the GABA_A receptor complex [171] (Fig. 1). Therefore, the pharmacological activation of peripheral-type BZP receptors by melatonin, with potential changes in neurosteroid production, provides another pathway for the pharmacological modulation of GABAergic activity by this hormone. Moreover, the ability of pharmacological concentrations of melatonin or BZPs to inhibit the adenylyl-cAMP pathway via putative G protein-coupled BZP receptors [172] suggests yet another neuropharmacological mechanism for modulation of GABAergic activity. It is known that relatively low doses of this hormone, which produce normal nocturnal levels, can also promote sleep in humans [91]. In contrast to the foregoing discussed data, the effects of these lower doses are not mediated by BZP receptors, as shown by the lack of antagonism by the central-type BZP antagonist flumazenil [173].

There is evidence that multiple kinases can phosphorylate various GABA_A receptor subunits, to alter GABAergic activity in the brain [174, 175]. Depending on the brain area and/or the receptor subunits involved, phosphorylation by various kinases can result in either activation or inhibition of GABAergic function. For example, tyrosine phosphorylation, induced by intracellular application of protein tyrosine kinase, has been found to increase GABA_A-mediated currents in cultured CNS neurons [176]. In contrast, protein kinase A-induced phosphorylation of the

GABA_A receptor is usually associated with GABA_A receptor desensitization in various CNS areas and results in decreased GABAergic activity [174]. Therefore, suppression of cAMP production by melatonin, which results in a decrease in protein kinase A-induced phosphorylation, may produce an opposite potentiating effect on GABAergic activity with enhancement of sleep induction. Ca²⁺/calmodulin-dependent kinase II, which is inhibited by physiological concentrations of melatonin [79], can phosphorylate and modify GABA_A receptor function [177]. Various PKC isozymes have been reported to play critical roles in the regulation of GABA_A receptor function and trafficking [178]. Moreover, PKC-induced phosphorylation of GABA_A subunits can alter the sensitivity of this receptor complex to allosteric modulators including neurosteroids. Thus, the possible activation of the PKC pathway by melatonin via the MT₁ receptor [107, 108] suggests an interplay between melatonin and neurosteroids in modulating GABAergic activity and sleep (Fig. 1).

6 Conclusions

Nearly all neurons in the SCN contain GABA, and GABA-driven mechanisms have been shown to play a key role in circadian coupling among regions and neurons in the SCN. Results are reviewed indicating that among the neurochemical effects of melatonin in the SCN, its interaction with the GABAergic system appears to be significant for coupling to sleep mechanisms. The clinical pharmacological significance of this interaction is illustrated by the recent approval of a prolonged release 2-mg melatonin preparation (CircadinTM) by the European Medicines Agency (EMA) as a monotherapy for the short-term treatment of primary insomnia in patients who are aged 55 or over [179].

Several melatonin derivatives have been shown to increase non-REM sleep in experimental animals and are also of potential pharmacological importance. So far, only one of these melatonin derivatives, ramelteon, has received approval from the USA Food and Drug Administration to be used as a sleep promoter [180]. Ramelteon is a nonspecific MT₁/MT₂ melatonergic agonist that has effects on melatonin receptors in the SCN and is effective in promoting sleep in experimental animals like cats and monkeys. In clinical trials, ramelteon reduced sleep onset latency and promoted sleep in patients with chronic insomnia, including an older adult population. Another compound, agomelatine, a novel melatonergic antidepressant with MT₁/MT₂ melatonergic agonism and 5-HT_{2C} serotonin receptor antagonism, has been recently approved by EMA and has been shown to improve sleep in depressed patients and to have an antidepressant efficacy that is partially attributed to its effects on sleep-regulating mechanisms [181]. To what extent ramelteon or agomelatine promotes sleep by regulating the sleep/wake rhythm through GABAergic mechanisms is presently unknown.

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GABA Involvement in the Circadian Regulation of Sleep

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Abstract γ -aminobutyric acid (GABA) plays a fundamental role in the circadian regulation of behavior and physiology. GABA and its receptors are found in most, if not all, cells of the suprachiasmatic nucleus (SCN), the location of the putative master circadian pacemaker. Furthermore, nearly every cell in the SCN receives GABAergic input. Thus, GABA is anatomically positioned to play a major role in regulating the circadian clock. The available data indicate firstly that GABA release in the SCN is required for nonphotic stimuli to phase shift the clock during the day, when the circadian clock is not reset by light. Secondly, the acute release of GABA in the SCN potentially inhibits the phase-shifting effects of light exposure at night, and these effects are likely mediated at least in part by extrasynaptic GABA_A receptors. Thirdly, several lines of recent evidence suggest that the sustained release of GABA in the SCN may mediate the ability of light to phase shift the clock. In summary, although much remains to be learned about GABA function in the SCN, existing data indicate that GABA activity in the SCN can profoundly influence circadian phase and the ability of phase shifting stimuli such as light to reset the clock.

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1 Introduction

Circadian rhythms provide a critically important temporal framework for nearly all behavioral and physiological functions. In the natural environment, these rhythms are synchronized by external cues (primarily the daily light–dark (LD) cycle) through the process of entrainment, resulting in a period of precisely 24 h and a fixed phase relationship between behavior and the light schedule [1–3]. Circadian clocks generate rhythms that are slightly longer or shorter than 24 h. The 24 h LD cycle synchronizes the clock so that all rhythms under its control exhibit a 24-h pattern. That light synchronizes circadian rhythms is best illustrated by the way in which brief light pulses phase shift circadian rhythms (for a review see [3]). Depending on when during the circadian cycle an organism is exposed to light, the result may be an advance, delay, or no effect on the phase of circadian rhythms. A light pulse given around the beginning of the daily active phase (i.e., CT 12–14) or 6–8 h after the beginning of the active phase (i.e., CT 18–20) produces a phase delay or phase advance in the circadian rhythm, respectively; whereas a light pulse given at any other time does not phase shift the clock.¹ Light pulses given in a 24-h cycle can therefore reset the phase of a circadian clock (i.e., advance or delay) on a daily basis so that rhythms under its control express a 24-h cycle.

The suprachiasmatic nucleus of the hypothalamus (SCN) is a putative circadian clock [4–7]. The SCN has a central role in the generation of circadian rhythms and their synchronization with the 24-h day–night cycle. There are several major SCN afferents including: the retinohypothalamic tract (RHT), which is a direct projection from the retina [8–11], the geniculohypothalamic tract (GHT), which is a direct projection from the intergeniculate leaflet (IGL) of the thalamus [12–15], and a direct projection from the median raphe of the midbrain [16, 17]. The RHT appears to be the most important pathway for communicating photic information to the SCN. Its destruction eliminates the ability of an animal to entrain to the LD cycle [18]. There is considerable evidence that glutamate serves as the primary neurotransmitter in the RHT, although other neurochemical signals may also be released from RHT terminals (for a review see [19, 20]). In fact, administration of glutamate agonists (e.g., NMDA) into the SCN mimics the phase-shifting effects of light [21], and glutamate antagonists block the phase-shifting effects of light [15, 22].

¹*Zeitgeber time* (ZT) is a standard way of indicating time within a circadian cycle entrained to a LD cycle with the onset of the dark phase arbitrarily defined as ZT 12 for nocturnal organisms.

Circadian time (CT) is a standard way of indicating time within a circadian-cycle exhibited in the absence of environmental time cues, with the onset of locomotor activity arbitrarily defined as CT 12 for nocturnal organisms.

2 Intra-SCN Communication

Dramatic progress has been made in understanding the molecular mechanisms involved in clock processes within the SCN. Individual neurons within the SCN exhibit circadian rhythmicity [23] as the result of interacting transcriptional and translational feedback loops in which Period (Per) genes play a role of critical importance (for reviews see [24, 25]). Expression of the products of these genes exhibits circadian rhythmicity and has been used experimentally to assess phase of the clock. However, not all SCN neurons display rhythmic clock gene expression [26–28], and SCN neurons are heterogenous in other important respects as well [23, 29–32] (see below). SCN neurons also exhibit a variety of different phases and free-running periods [30, 32, 33]. In summary, the timekeeping mechanisms in the SCN appear to be composed of a heterogenous population of neurons, only some of which are capable of spontaneous circadian rhythmicity. Therefore, communication among individual SCN neurons is critical for circadian time-keeping. Much remains to be learned about how neurons communicate within the SCN. There are a number of different types of signaling processes that may be responsible for communication between SCN neurons (e.g., gap junctions [34, 35]; see [36] for a review); however, it seems likely that synaptic activity plays a key role in this communication and in the coupling of circadian oscillators in the SCN [31, 37–40].

3 GABA in the SCN

γ -aminobutyric acid (GABA) [41–44], its receptor [45–47], synthesis enzyme [48–50], and transporter [51] are found in most, if not all, cells of the SCN. Electrophysiological evidence indicates that tonic GABAergic input, mediated by the GABA_A receptor, is received by nearly every SCN cell [52, 53]. The source of GABA appears to originate from both within and outside of the SCN [54, 55]. As such, it is very likely that GABA plays a fundamental role in the SCN and there is substantial evidence from studies conducted both *in vivo* and *in vitro* in support of this view.

Recently, it has been reported by several groups that in addition to GABA's well known inhibitory effects [56–58], it can also have excitatory effects under certain conditions in the SCN [38, 52, 59–61]. Some investigators report that GABA is excitatory during the subjective day [60, 61] and others report that GABA is excitatory during certain parts of the subjective night [52, 59]; still others report that GABA is exclusively inhibitory [56–58]. There are a number of possible reasons for these observed differences in results [60]. However, whether GABA can be excitatory under certain conditions or whether it is exclusively inhibitory, there is no question that GABA plays a critical role in regulating neuronal activity and excitability in the SCN [52, 58, 60, 61].

4 Role of GABA in the SCN

Cells in the SCN exhibit a synchronized circadian rhythm in spontaneous neural activity *in vivo* [62]. Individual SCN neurons retain the ability to generate spontaneous rhythms in electrical activity when isolated in culture, but these individual rhythms drift out of phase with each other [23]. Until recently, GABA was thought to play an important role in the synchronization of individual pacemaker neurons within the SCN. Daily pulses of GABA administered in culture can synchronize the rhythms in spontaneous electrical activity exhibited by isolated SCN neurons [31]. However, more recently, it has been shown that long-term inhibition of endogenous GABA activity *in the SCN slice* does not interfere with the ability of individual neurons to synchronize daily rhythms in clock gene expression [39]. That is, peak *Per2* bioluminescence exhibited by individual neurons within a slice from *PERIOD2::luciferase* mice continued to occur at a similar time each day following exposure to a combination of GABA_A and GABA_B antagonists for 10 days. These data indicate that, although GABA may be *sufficient* to synchronize cellular activity in the SCN, it is not *necessary*.

5 GABA and Photic Entrainment

Given its ubiquity in the SCN, it seems likely that GABA might modulate the phase-shifting effects of light. In fact, GABA profoundly inhibits the ability of light to phase shift the circadian clock in both nocturnal and diurnal rodents. For example, microinjections of the GABA_A agonist muscimol or the GABA_B agonist baclofen into the SCN region of hamsters [63, 64], or grass rats [64, 65], just prior to a light pulse in the early subjective night inhibit light-induced phase delays. Conversely, microinjections of the GABA_A antagonist bicuculline or the GABA_B antagonist CGP-35348 into the SCN region of hamsters before a light pulse in the early subjective night enhance light-induced phase delays [63, 64]. Interestingly, microinjection of muscimol or baclofen (GABA_A and GABA_B agonists, respectively) into the SCN region of hamsters reduces the phase advancing effects of light in the late subjective night, but microinjection of bicuculline (GABA_A antagonist) or CGP-35348 (GABA_B antagonist) does not increase the phase shifting effects of light during the late subjective night [64]. In other studies, the microinjection of GABA_A and GABA_B agonists and antagonists into the SCN were found to modulate the ability of light to induce c-Fos immunoreactivity in a manner very similar to their effects on light's ability to induce phase shifts [66]. Taken together, these data indicate that the acute release of GABA in the SCN can have potent inhibitory effects on the ability of light to induce phase shifts during the subjective night. In fact, these inhibitory effects of GABA are so potent that high levels of GABA release would have the potential to block the ability of an individual to entrain to the LD cycle. Interestingly, these data provide indirect evidence that GABA may be

released in association with light exposure. The ability of GABA antagonists to enhance the phase-delaying effects of light during the early night suggests that GABA is released either spontaneously or in association with light presentation at this phase of the circadian cycle. In contrast, the inability of GABA antagonists to enhance the phase-advancing effects of light during the late night suggests that GABA is not released either spontaneously or in association with light presentation at this phase of the circadian cycle.

6 GABA and Nonphotic Entrainment

Single acute injections of GABA_A or GABA_B agonists into the SCN region of nocturnal or diurnal rodents do not mimic the phase-shifting effects of light. When GABA agonists are injected into the SCN during the early or late subjective night (e.g., when light produces phase delays or advances, respectively), little or no phase shifting occurs [63–65]. However, when the GABA_A agonist muscimol is injected into the SCN during the subjective day, when light does not produce phase shifts, large phase shifts occur. In hamsters, which are nocturnally active, muscimol produces large phase advances [67–69], while in grass rats, which are diurnally active, muscimol produces large phase delays [65, 70].

There are a number of stimuli other than light that can entrain the circadian clock. These nonphotic stimuli generally involve an increase in arousal state. Examples include spontaneous running on a novel wheel, several hours of gentle handling, and saline injections. The phase-shifting effects of nonphotic stimuli occur primarily in the day portion of the circadian cycle, when light does not produce phase shifts. GABA release in the SCN appears to be involved in mediating the effects of these nonphotic stimuli. This is evidenced by the ability of GABA agonists to mimic nonphotic shifts. Phase shifts are induced by the activation of GABA_A receptors *in vivo* during the subjective day (Fig. 1) [67, 69, 71]. In nocturnal animals, this phase-advancing effect of GABA peaks during the mid-day, and it is similar to advances produced by behavioral stimuli [72, 73]. Thus, GABA_A receptor activation seems sufficient to induce nonphotic-like shifts.

Recent data suggest that many, if not all, nonphotic stimuli that produce phase shifts are communicated to the SCN by the GHT. The GHT produces both GABA and NPY. Injection of NPY or GABA agonists into the SCN can mimic the phase-shifting effects of nonphotic stimuli. In addition, the phase-shifting effects of nonphotic stimuli are attenuated by the injection of NPY antisera into the SCN. Thus, NPY release seems to be part of this nonphotic pathway. However, these effects of NPY are completely blocked by the GABA_A antagonist bicuculline injected into the SCN *in vivo* [69] and *in vitro* [74]. This suggests that GABA_A receptor activation in the SCN is necessary for the induction of these nonphotic shifts. The involvement of GABA in both photic and nonphotic entrainment suggests that GABA receptor activation may play a critical role in the clock-setting effects of environmental stimuli.

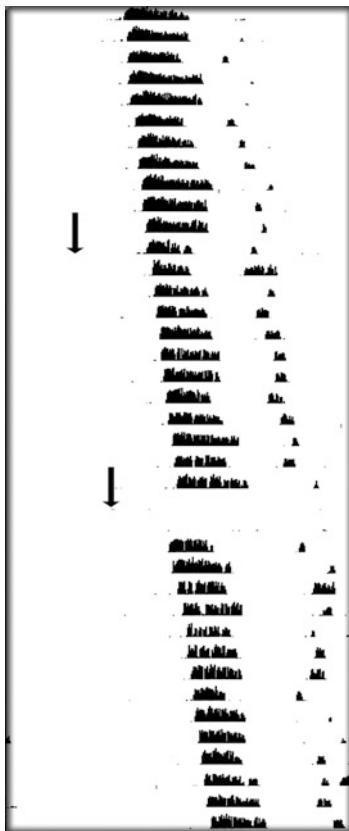


Fig. 1 GABA_A receptor activation in the suprachiasmatic nucleus (SCN) during the mid-day: Circadian rhythmicity in the spontaneous wheel running of a Syrian hamster is depicted graphically in this actogram. Each *horizontal line* represents a 24 h day, with each *dark bar* representing the number of wheel revolutions in 10-min intervals. The animal is in constant darkness, which allows the endogenous period of its circadian clock to be expressed. Note that the onset of wheel-running occurs later each day, indicating an endogenous rhythm greater than 24 h. *Arrows* represent the time of treatment by intracranial microinjection near the SCN. Saline (*top arrow*) has little effect on the activity rhythm. However, the GABA_A receptor agonist muscimol (*bottom arrow*) has a profound effect on the circadian rhythm of wheel-running activity. Onset following treatment occurs about 90 min earlier when compared to the days before treatment. This earlier onset is a phase advance in the circadian rhythm. The stability of this phase advance is apparent in the subsequent activity bouts, which continue from the first shifted activity onset with the same endogenous period

7 GABA and Phase Shifts

Understanding how light entrains the circadian clock contained in the SCN requires a better understanding of the key processes that occur between exposure to light and the phase shift produced in the clock. This time interval can be separated into two

stages: a *transient response*, during which light rapidly induces a series of events that occur on the order of seconds to minutes, and a *sustained response*, during which the transient core response to light induces activity in a subset of neurons that begins approximately 1 h after the light pulse; it appears that this secondary sustained response is necessary to produce a phase shift in the circadian clock.

During the transient response, light exposure initiates a number of events, including NMDA receptor activation, which serves to increase spike frequency in the ventrolateral “core” region of the SCN.² Cells in this region contain vasoactive intestinal polypeptide and are densely innervated by retinal afferents. These events occur quickly as evidenced by the fact that brief pulses of light and acute injections of NMDA each produce large phase advances and delays [21, 78].

The transient response to light is followed by a sustained response in the dorsomedial or “shell” region of the SCN. The most complete analysis of the sustained response comes from studies in hamsters given 30 min light pulses at times that produce phase advances [26] and from transgenic mice given 30 min light pulses at times that produce phase delays or advances [79]. In hamsters, Per 1 was induced within 60 min of the light pulse and remained elevated for approximately 4 h. In transgenic mice, it was possible to examine the effects of light on both electrical activity and Per 1 gene activity (with a fluorescent reporter) by providing a light pulse and then preparing an SCN slice 1.5 h later. This approach also demonstrated that brief light exposure results in a sustained response lasting several hours.

Recent evidence suggests that a sustained release of GABA in the SCN may communicate information from neurons transiently responding to light to neurons that form the clock [60]. Albus et al. (2005) demonstrated that both the dorsal and ventral regions of the SCN slice exhibit a comparable bimodal rhythm in spontaneous electrical activity following a 6-h delay in the LD cycle of rats. One of these rhythms is fully phase-shifted by the light cycle and one is entirely unaffected by the shift. Horizontal cuts separating the dorsal and ventral regions of the SCN resulted in two distinct, yet out of phase, rhythms in electrical activity. While the rhythm in neural activity within the ventral region exhibited an immediate delay, the rhythm in the dorsal SCN remained unshifted. Interestingly, the continuous application of a GABA_A antagonist to an intact SCN slice immediately following a 6-h delay in the LD cycle produced the same result, with the rhythm in the ventral SCN exhibiting a single peak fully reentrained to the new LD cycle and the rhythm in the dorsal SCN unaffected by the shift [60]. These data are consistent with the hypothesis that a sustained GABAergic signal is required to communicate phase

²The core/shell model is an oversimplification of SCN organization [75–77]. While it is clear that functional subdivisions exist within the SCN, it is also clear that early generalizations about the core/shell organizational concept are not entirely correct. For example, detailed analysis of retinal innervation patterns, cell phenotypes, as well as other anatomical and functional markers have correctly led to the view that the core and shell distinction is artificially restrictive, particularly when viewed across species. For the sake of simplicity, we will use the core/shell terminology as a way of indicating that there are functionally distinct subdivisions within the SCN.

shift information between the retinorecipient cells in the ventrolateral region and the endogenously rhythmic cells in the dorsomedial region of the SCN.

If a sustained release of GABA within the SCN mediates the phase shifting effects of light, then one would expect that sustained administration of GABA agonists to the SCN would mimic the phase shifting effects of light. Preliminary data from our lab demonstrate that the GABA_A agonist muscimol can produce large phase delays similar to the phase-delaying effects of light. In these experiments, we examined whether a sustained 4-h administration of muscimol was sufficient to induce phase shifts. Hamsters were implanted with guide cannula aimed at the SCN and allowed to establish a stable circadian wheel-running rhythm in constant darkness. During the beginning of their active period (90 min after activity onset), hamsters received a series of four injections at 1-h intervals containing either vehicle (saline) or muscimol (2.5 mg/ml; 100 nl/injection). All injection sites were histologically confirmed at the end of the experiment. As can be seen in Fig. 2, four injections of muscimol induced large phase delays (>1.3 h shifts) that were significantly greater than vehicle. We went on to demonstrate that muscimol only induced phase delays when it was administered over a 4-h interval. When muscimol was administered over a 1-, 2-, or 3-h interval, no phase delays were observed (Fig. 3). Further studies ruled out the possibility that the phase of administration during this 4-h injection interval was critical for the induction of phase delays. Further, we saw a significant difference in the magnitude of the phase delay depending on the dose administered (Fig. 4). These data demonstrate that the sustained administration of a GABA_A agonist into the SCN can mimic the phase-delaying effects of light. Together, this evidence strongly supports the hypothesis that the sustained nature of GABA_A receptor activation is the critical variable resulting in phase shifts of the circadian clock during the night.

8 GABA_A Receptor Subunits in SCN

Several investigations have examined the distribution of GABA_A receptor subunits in the SCN. For many of these subunits, there are consistent findings across techniques and species. However, for other subunits, there is still disagreement. Therefore, we do not have sufficient evidence to identify the most likely combinations of subunits in the SCN. The proteins α_2 and α_5 are the subunits most frequently found in the SCN. Protein and mRNA has been detected in the rat, mouse, and hamster [45–47, 79]. Presence of the remaining α subunits in the SCN of rodents, however, has not been convincingly demonstrated. α_1 mRNA has been found in mouse SCN dissections [47] and protein in the hamster by immunohistochemistry [79], albeit in low levels. α_3 and α_4 mRNA is also found in the mouse. However, α_4 has not been confirmed by any other studies, and α_3 remains questionable because protein, but not mRNA [80], is found in the rat [45].

β_1 protein [46] and mRNA [47] has been localized to the SCN of hamsters. Interestingly, this subunit was found to be rhythmic in the SCN and median

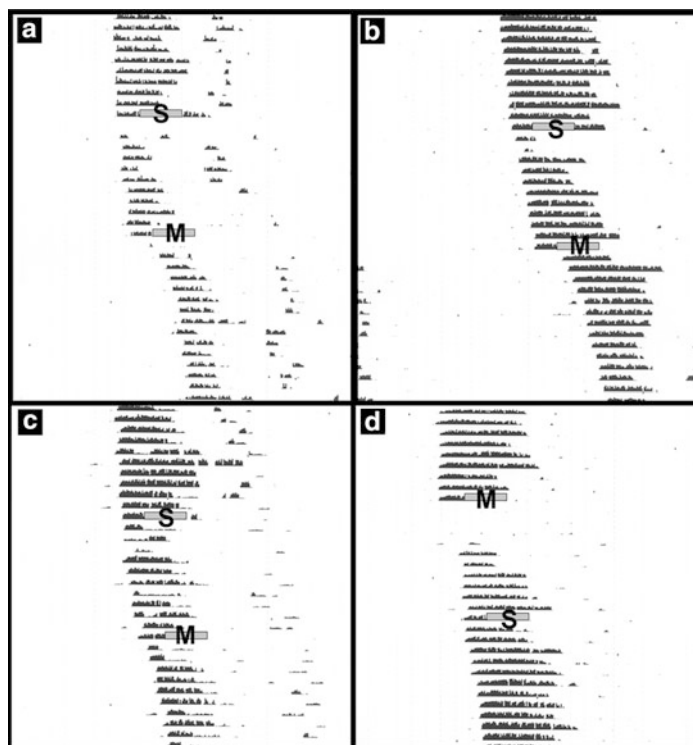


Fig. 2 The effect of a sustained (4 h) administration of the GABA-A agonist muscimol: Circadian rhythmicity in the spontaneous wheel running of a Syrian hamster is depicted graphically in this actogram. Each horizontal line represents a 24 h day, with each dark bar representing the number of wheel revolutions in 10-min intervals. Muscimol was delivered in four injections at 1 h intervals beginning at CT 13.5. Gray bars denote the 4-h injection period. Abbreviations: *M* muscimol; *S* saline; *A–D* individual hamsters

eminence, with peak protein expression during the night. Beyond the obvious implication in the rhythmic sensitivity to GABAergic drugs, this finding raises the possibility that other GABA_A subunits in addition to β_1 may be rhythmic. In the above investigation, α_2 also showed a nonsignificant trend toward rhythmicity, while the β_2 and β_3 did not [46]. Some of the controversy surrounding the presence of certain subunits in the SCN, therefore, may be the consequence of variations in collection time.

mRNA for the β_2 and β_3 proteins has also been detected in the SCN [47]. However, this could not be confirmed by immunohistochemistry using a β_2/β_3 antibody [45], leading the authors to propose that a difference in technique was responsible for the different findings. It was suggested that during dissection, tissue surrounding the SCN known to be rich in β_2 and β_3 may have been inadvertently included [45]. Recently, this argument was weakened by relatively high immunohistochemical labeling of β_2/β_3 protein in the hamster SCN [79]. The existence of the β_2 and β_3 subunit in the SCN requires further investigation.

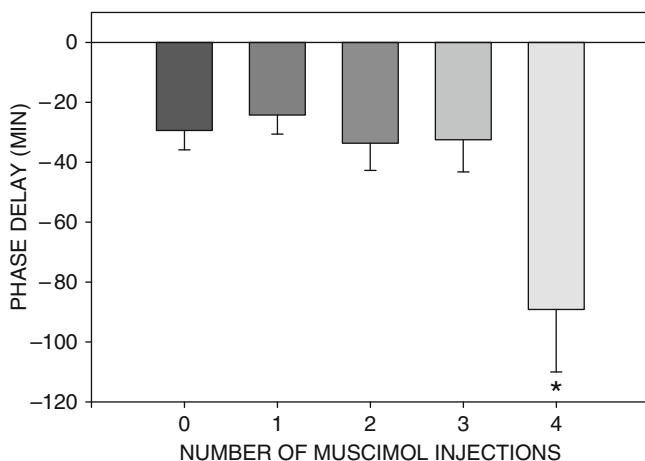


Fig. 3 Phase delays produced by injection of different durations of muscimol into the SCN of hamsters housed in constant darkness beginning at ct 13.5. No less than four consecutive injections were required for GABA_A receptor activation to induce phase shifts during the night. Each animal received four injections. Animals that received less than four injections of muscimol were given saline to bring the total number of injections to four. Muscimol injections were always given first. Bars represent mean \pm SE. *, significantly different than all other groups

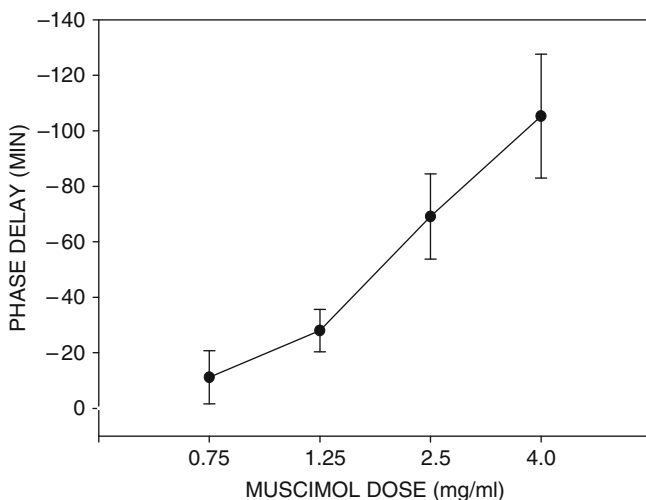


Fig. 4 Dose response curve for the sustained 4 h administration of a GABA_A receptor agonist. Mean \pm SE of phase delays produced by four microinjections of muscimol. Treatment was given in the early night to hamsters housed in constant darkness

Investigation of the γ subunit has proven to be relatively straightforward. Both γ_1 and γ_2 mRNA are found in SCN tissue dissected from the mouse [47]. mRNA and protein are also detected by the more anatomically specific methods of in situ

hybridization and immunohistochemistry [45, 80]. The subunits omitted from discussion here have not yet been investigated, with the exception of a single study, which failed to find δ subunit mRNA in the mouse SCN [47]. We are currently unaware of available information concerning the existence of γ_3 , ϵ , θ , or π subunits in the rodent circadian clock.

We recently became interested in the circadian functions of specific receptor subunit assemblies possessed by the SCN. Because the combination of subunits determines the pharmacological profile and/or cellular location of the GABA receptor [81–84], we hypothesized that different subtypes of GABA_A receptors in the SCN are responsible for the multiple actions of GABA. Our previously discussed findings regarding the sustained administration of muscimol led us to focus on a subtype of GABA_A receptors possessing the δ subunit. This subunit is expressed in most brain regions but found primarily in areas of the hippocampus, cortex, and thalamus [47, 85–88]. Evidence suggests that incorporation of this protein is responsible for directing the receptors to extrasynaptic locations, where they are believed to respond to tonic levels of GABA in the extracellular space [89]. We hypothesized that our sustained application paradigm may simulate an increase in tonic GABA levels and allow activation of these receptors. We chose a pharmacological approach to investigate this possibility. THIP (4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol) is a GABA_A receptor agonist that shows higher efficacy than GABA at δ subunit-containing receptors. This allowed us to preferentially activate receptors containing the δ subunit and determine its role in phase-shifting the circadian clock.

In this study, we injected THIP into the SCN of hamsters to investigate the role of these extrasynaptic receptors in GABA's action on the circadian system [90]. Our findings lend support to the hypothesis that GABA acts through multiple configurations of receptor in the SCN to exert complex effects on circadian timing. THIP microinjected during the day did not phase shift wheel running rhythms, while at night, the agonist abolished light-induced phase shifts in wheel running activity and light-induced expression of *Per1* and *Per2* mRNA (Figs. 5 and 6) [90]. These findings are consistent with the hypothesis that a subtype of GABA_A receptors selectively sensitive to THIP inhibits the ability of light to reset circadian timing, while a separate population of GABA_A receptors, insensitive to THIP, is responsible for the ability of GABA to cause phase shifts during the daytime. Thus, THIP seems to be unique among GABA_A receptor agonists in its ability to inhibit the effects of light at night without direct phase-altering effects during the subjective day.

We also sought to determine if the actions of THIP were due to presynaptic inhibition of glutamatergic input to the SCN. It appears that the effects of light in the SCN are due to the activation of NMDA type glutamate receptors by glutamate released from terminals of the retinohypothalamic tract. Thus, THIP could block light-induced phase shifts by preventing glutamate release from RHT terminals. To test this possibility, we examined whether THIP blocked the ability of NMDA to produce phase shifts. As can be seen in Fig. 7, THIP significantly reduced the effects of NMDA microinjected into the SCN [90]. The finding that both photic and

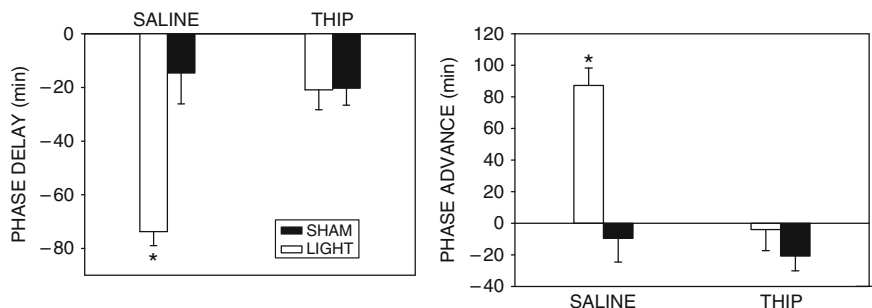


Fig. 5 THIP inhibits light-induced behavioral phase shifts in the subjective-night. THIP (a GABA_A superagonist at δ subunit receptors) delivered to the SCN region just prior to a light pulse during the early subjective night (CT 13.5) inhibited phase delays so that they were not different from saline treated control animals (*left*). THIP administered just prior to a light pulse in the late subjective night (CT 20) significantly inhibited the resulting phase advances (*right*). In both the early and late night, the effect of light in saline treated animals was significantly greater than all other groups. Bars represent mean \pm SEM; *, statistically significant difference

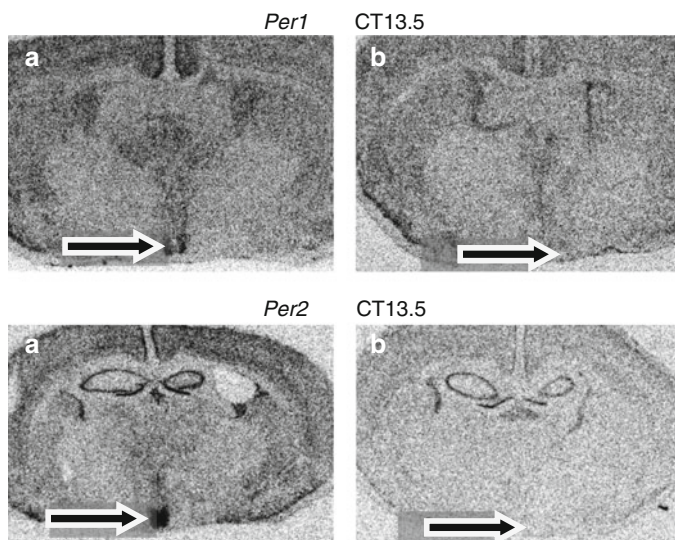
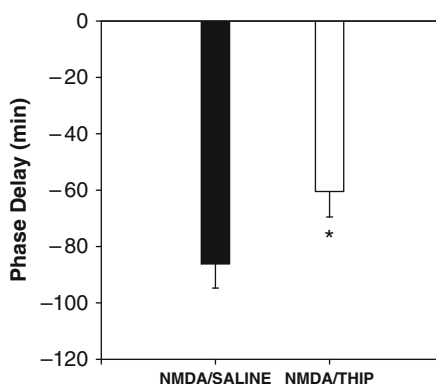


Fig. 6 THIP inhibits light-induced Period mRNA expression during the subjective night. Representative autoradiograms demonstrate the *Per1* and *Per2* mRNA increases induced by light in the SCN during the early night (CT 13.5, **a**). These increases were abolished in animals pretreated with THIP (**b**). In situ hybridization for *Per1* and *Per2* mRNA was performed on brain sections from Syrian hamsters treated with drug or saline. Drug and saline treatments were immediately followed by a 15-min pulse of light. Arrows indicate the SCN

NMDA-induced phase shifts are inhibited by THIP indicates that the receptors activated by THIP exist on cells immediately postsynaptic to retinal terminals. However, the incomplete nature of the inhibition may indicate the involvement of

Fig. 7 Inhibition of NMDA-induced phase delays. NMDA microinjected into the SCN region induced phase delays in the circadian rhythm of wheel running activity. THIP delivered immediately before NMDA significantly inhibited these phase delays. *Bars* represent Mean \pm SEM; * $p < 0.05$



other receptors. Nevertheless, these data support the hypothesis that the δ subunit-containing receptors exist on retinorecipient cells of the SCN, downstream of glutamate release. Overall, our findings support the hypothesis that there are at least two distinct subpopulations of GABA_A receptors in the SCN, both sensitive to muscimol, but only one of which is sensitive to THIP. Further, it seems that the THIP sensitive subtype is involved in the ability of GABA to block light-induced phase shifts.

9 Conclusion

The most important functional properties of circadian clocks are their ability to maintain self-sustained rhythmicity and their ability to synchronize that rhythmicity with the day–night cycle. Given the strong evidence that the suprachiasmatic nucleus contains a circadian clock and that GABA is found within nearly every cell of this structure, it seems likely that GABA plays a fundamental role in clock function. While there is evidence that GABA can synchronize the cellular circadian oscillators in the SCN, the best evidence is that GABA is not necessary for coupling these oscillators into a functioning circadian pacemaker [31, 39]. On the other hand, there is substantial evidence that activation of GABA receptors within the SCN can have powerful effects on the mechanisms that govern entrainment of the SCN. Activation of GABA receptors appears to be capable of modulating the ability of both photic and nonphotic stimuli to reset the circadian clock in the SCN [63–65, 70, 74].

Interestingly, GABA appears to be capable of both inhibiting the ability of light to reset the clock when given acutely and of mimicking the ability of light to reset the clock when given in a more sustained manner. The powerful inhibitory effect of relatively brief activation of GABA_A and GABA_B receptors achieved by injection of GABA agonists in the SCN has been well characterized [63–65]. In addition, the ability of single injections of GABA receptor antagonists to enhance the phase-delaying effects of light during the early subjective night [63, 64] suggests that

GABA is endogenously released within the SCN at this time and that this release serves to reduce the magnitude of light-induced phase delays. Taken together, data employing GABA agonists and antagonists strongly support the hypothesis that acute release of GABA within the SCN inhibits the ability of light to reset the circadian clock.

By contrast, there is also evidence supporting the possibility that the sustained release of GABA in the SCN mediates the ability of light to phase shift the clock. There is now strong support for the possibility that brief light pulses produce a sustained activation (i.e., 4 h or longer) in a subpopulation of SCN neurons [26, 79]; and because GABA is found so extensively throughout the SCN, it seems likely that GABA would be released in response to this activation. Evidence that a sustained release of GABA could mediate at least some of the phase-shifting effects of light comes primarily from studies employing multiple injections of the GABA_A agonist muscimol into the SCN. Hourly injections of muscimol beginning during the early subjective night mimic the phase-delaying effects of light if given over a 4-h interval but not when administered over shorter intervals. There is also important evidence that the phase delays produced by the sustained administration of muscimol during the beginning of the subjective night are dose-dependent and that the duration of GABA administration is critical for the induction of phase delays while the phase of administration during this 4-h window is not. Despite the evidence that light pulses appear to produce a sustained neuronal activation in the SCN and that the sustained administration of GABA agonists can mimic the phase-delaying effects of light, a critical analysis of the hypothesis that the sustained release of GABA mediates the phase-shifting effects of light within the SCN awaits further study. In particular, it will be important to confirm that light pulses induce a sustained release of GABA and that the sustained but not the acute administration of GABA antagonists blocks the ability of light to phase shift the clock.

GABA also appears to play a critical role in nonphotic phase shifting. It seems likely that many, if not all, nonphotic phase shifting stimuli are communicated to the SCN through the release of NPY from terminals of the GHT. The existing evidence suggests that the phase-shifting effects of NPY are mediated by the release of GABA in the SCN and the resultant activation of GABA_A receptors in the SCN. This hypothesis is supported by the ability of GABA_A antagonists to block the ability of NPY to induce phase advances by its action in the SCN and by the ability of GABA_A agonists to mimic the phase-advancing effects of NPY [70, 72–74]. GABA has a complex role in controlling circadian phase by its actions in the SCN, and much remains to be learned about the role of GABA in both photic and nonphotic entrainment. For example, it will be critical to characterize more clearly GABA_A receptor subunits in the SCN and to determine how the expression of these subunits is regulated, including their possible rhythmicity. Even the limited amount of data described above strongly suggests that populations of GABA_A receptors composed of different combinations of subunits may have very different roles in circadian timekeeping. Another intriguing aspect of GABA function in the SCN that requires further clarification is whether GABA can be both excitatory and inhibitory. It will also be important to determine the events that occur downstream

from the activation of GABA receptors in the SCN. For these studies to be successful, it will be critical to determine whether different types of stimuli induce dramatically different durations of GABA release in the SCN and whether acute versus sustained release of GABA mediate different circadian functions.

Perhaps the most parsimonious hypothesis at present is that GABA release within the SCN represents a final common pathway to the clock within the SCN and that the temporal patterning of its release determines its effects on the clock's phase. Whether or not this view is correct, it is clear that manipulation of GABA activity in the SCN has the potential to profoundly affect the phase of the pace-maker and the ability of phase shifting stimuli such as light to reset the clock. Because many GABA-active drugs are used for a variety of sleep and psychiatric disorders, a better understanding of GABA function in the SCN will provide new opportunities for the development of more effective pharmaceutical therapies for a wide variety of illnesses.

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Part III

Hypnotics

Pathophysiology of Sleep Disorders

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Abstract Current concepts on neurobiological mechanisms underlying sleep disorders such as insomnia, narcolepsy, and restless legs syndrome/periodic limb movement disorder are being reviewed in this chapter, which includes short discussions of clinical key features, diagnostic criteria, and therapeutic aspects of these disorders. Chronic insomnia is a common and complex 24-h disorder that derives from a multifactorial interaction of biological and psychological factors affecting both sleep and wakefulness. Several models of the pathophysiology of insomnia have been elaborated, but no single underlying pathophysiological process has been shown to represent a causal factor. Importantly, persistent insomnia has been identified as a risk factor for the development or exacerbation of certain psychiatric conditions. Narcolepsy is characterized by excessive daytime sleepiness and cataplexy. Its pathophysiology is largely associated with hypocretin ligand deficiency possibly caused by the postnatal loss of hypocretin-containing neurons. An immune-mediated etiology for hypocretin deficiency has been suggested. Sleep-related movement disorders include the restless legs syndrome (RLS) and periodic limb movement disorder (PLMD). Pharmacological, neuroimaging, and electrophysiological studies suggest a dysfunction of the supraspinal inhibitory system triggering RLS and PLMD. In addition, recent studies suggest a strong genetic contribution to both disorders. Further research on sleep disorders is expected to guide new treatment strategies.

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1 Insomnia

The word “insomnia” is derived from the Latin prefix “in-” meaning “lacking” and the Latin noun “somnus” meaning sleep, and is often translated as sleeplessness. However, the term insomnia comprises far more than just the difficulty of falling or staying asleep, which in clinical and sleep medicine as well as medical science is referred to as sleep-onset and sleep-maintenance insomnia.

1.1 Key Features

The clinical picture of insomnia partly depends on its cause, manifestation, and duration. Generally, the presenting complaints are sleep disturbances such as difficulty falling or staying asleep or early-morning awakening. Additionally, patients describe their sleep as nonrestorative and complain about feeling unrefreshed after sleeping. But complaints of insomniacs do not concern the nighttime alone. Further clinical features are daytime tiredness and self-perceived reduced daytime functioning. Noteworthy, patients with insomnia typically underestimate their performance and overestimate their impairment [1].

1.1.1 Diagnostic Criteria and Subtypes of Insomnia

To diagnose primary insomnia, the diagnostic criteria defined in the International Classification of Sleep Disorders, second edition, (ICSD-2), published by the American Academy of Sleep Medicine (AASM) [2], must be met, as exemplified in Table 1.

Besides insomnia as an independent disorder (primary insomnia), comorbid insomnia (or synonymously secondary insomnia) is a common symptom in many psychiatric and somatic diseases [3, 4] (see Table 2).

Furthermore, insomnia can also occur as a side effect of medication, e.g., [3, 4]. In addition to differentiating between the cause (primary, comorbid) and manifestation (difficulty initiating or maintaining sleep, early awakening, or nonrestorative sleep) of insomnia, discrimination can further be made by considering the duration of insomnia as acute or chronic. To consider similarities and differences of insomnia with regard to different causes, manifestations, and durations, the ICSD-2 [2] specifies eleven subtypes of insomnia (Table 3) which all meet the general criteria of insomnia.

1.1.2 Comorbid Insomnia and Major Depressive Disorder

With regard to pathophysiological issues, insights into the mechanisms of disorders that regularly go along with comorbid insomnia might lead to a better understanding of the pathophysiology of primary insomnia. This is especially true for major

Table 1 General criteria of insomnia [2]

A.	A complaint of difficulty initiating sleep, difficulty maintaining sleep, waking up too early, or sleep that is chronically nonrestorative or poor in quality. In children, the sleep difficulty is often reported by the caretaker and may consist of observed bedtime resistance or inability to sleep independently
B.	The above sleep difficulty occurs despite adequate opportunity and circumstances for sleep
C.	At least one of the following forms of daytime impairment related to the nighttime sleep difficulty is reported by the patient: <ol style="list-style-type: none">1. Fatigue or malaise2. Attention, concentration, or memory impairment3. Social or vocational dysfunction or poor school performance4. Mood disturbance or irritability5. Daytime sleepiness6. Motivation, energy, or initiative reduction7. Proneness for errors or accidents at work or while driving8. Tension, headaches, or gastrointestinal symptoms in response to sleep loss9. Concerns or worries about sleep

Table 2 Comorbid insomnia

Somatic condition	References
Metabolic	
Diabetes	[193]
Dermatological	
Psoriasis	[194]
Pulmonary	
Asthma	[195]
COPD	[196]
Gastrointestinal	
Gastroesophageal reflux disease	[197]
Musculoskeletal	
Sjögren’s syndrome	[198]
Osteoarthritis	[199]
Rheumatoid arthritis	[200]
Fibromyalgia	[195, 198, 200]
Osteoporosis	[195]
Renal	
Chronic kidney disease	[201, 202]
Cardiovascular	
Coronary artery disease	[203, 204]
Hypertension	[205–207]
Other	
Menopause	[208]
Cancer	[208]
Acute viral illnesses	[208]
HIV	[208]
Lyme’s disease	[208]

Table 3 Subtypes of insomnia [2]

1.	Adjustment insomnia (acute insomnia)
2.	Psychophysiological insomnia
3.	Paradoxical insomnia
4.	Idiopathic insomnia
5.	Insomnia due to mental disorder
6.	Inadequate sleep hygiene
7.	Behavioral insomnia of childhood
8.	Insomnia due to drug or substance
9.	Insomnia due to medical condition
10.	Insomnia not due to substance or known physiological condition, unspecified (nonorganic insomnia, NOS)
11.	Physiological (organic) insomnia, unspecified

depressive disorder (MDD), which is very often accompanied by comorbid insomnia and shares objective findings, e.g., alterations in the neuroendocrine system, with primary insomnia.

1.2 Pathophysiological Concepts

Although, as shown above, primary insomnia is diagnosed solely by clinical criteria, distinct alterations of objective measures can be found that might, on the one hand, help to make a diagnosis and choose the right treatment and, on the other, are expected to help to develop new treatment strategies by giving further insight into possible pathophysiological mechanisms.

1.2.1 Neuroendocrine Parameters

As mentioned above, it is not only that comorbid insomnia is a common symptom in MDD, e.g., [5] and insomnia often precedes full-blown MDD, e.g., [6, 7], but primary insomnia can also go along with changes in neuroendocrine systems, especially the hypothalamic–pituitary–adrenal (HPA) axis [8], which also appear in MDD.

Hyperactivity of the HPA axis is a well-known phenomenon in MDD, e.g., [9], and is typically reflected by elevated cortisol levels, e.g., [10], and abnormal HPA axis regulation as indicated by the dexamethasone test or the dexamethasone-CRH test [11]. As in MDD, the HPA axis seems to be overactive in many patients with primary insomnia, as concluded from elevated urinary cortisol levels [12, 13]. However, there are also contradictory results showing no increased cortisol secretion in patients with insomnia [14]. Furthermore, peer-reviewed results from dexamethasone tests or dexamethasone-CRH tests in primary insomnia are still lacking. It is assumed that HPA axis hyperactivity in MDD is caused by increased corticotropin-releasing hormone (CRH) activity [15]. CRH hyperactivity has also been

considered to play a major role in primary insomnia [8, 16, 17]. It has been hypothesized that CRH hyperactivity results from genetic predispositions or environmental factors, such as early stress experiences, and leads to excessive CRH responses to stress, which in turn directly activate the locus coeruleus (LC) and, over time, indirectly affect the hippocampus (HC) by sustained elevated glucocorticoid (GC) levels [16, 17]. Moreover, the use of glucocorticoid receptor or CRH receptor antagonists and mineralocorticoid receptor agonists has been considered for treating insomnia [8].

1.2.2 Insights from Polysomnography, Actigraphy, Electroencephalography, and Multiple Sleep Latency Test

As stated above, polysomnographic parameters are no criteria for diagnosing insomnia. Instead, polysomnography (PSG) has even been regarded to be of little use for objectifying the presence of insomnia [18, 19], and former as well as current recommendations are that polysomnography is not indicated in routine evaluation of insomnia except for excluding a suspected sleep-related breathing disorder, a suspected periodic limb movement disorder, or certain other indications [20–23]. However, polysomnography in insomnia plays a role in clinical trials, especially in treatment efficacy studies [24–26]. Here, typical objective polysomnography measures of interest are wake after sleep onset (WASO), latency to persistent sleep (LPS), sleep efficiency (SE), number of awakenings (NAW or NUMA), wake time during sleep (WTDS), and slow-wave sleep (SWS). Typical findings in primary insomnia are prolonged sleep onset latency (SOL, equivalent to LPS), increased wake time after sleep onset, reduced sleep efficiency [27], and reduced slow-wave sleep [28]. However, as presented above, insomnia is a heterogeneous disease. Hence, the quantity and extent of altered PSG measures are subject to variation between individuals. Furthermore, drug-induced changes of PSG measures are also seen in healthy subjects and therefore are not appropriate biomarker of treatment response in insomnia.

In addition to PSG, actigraphy is a method to assess data about day- and nighttime activity and obtain sleep-related objective measures. Although there exist some reports about the use of actigraphy in insomnia, its role in the diagnosis of insomnia is discussed controversially [29–36]. Besides the controversy as to whether actigraphy is a valuable diagnostic tool in insomnia, results from actigraphy have not contributed to a better understanding of the underlying pathology of insomnia nor have they helped to distinguish subtypes of patients with insomnia so far. However, the evidence of underlying pathophysiological mechanisms is obtained from sleep electroencephalography (EEG) recordings in the form of spectral analysis. Sleep EEG spectral analysis differs from classical sleep EEG analysis by not making use of the Rechtschaffen and Kales criteria of visual sleep stage classification [37] but of EEG frequency analysis independent from sleep stages. A common finding in the sleep EEG of patients with insomnia is an increased amount of beta activity during the sleep onset period (SOP) [38] and

during NREM sleep [39]. Interestingly, this correlates with sleep complaints in patients with subjective insomnia (relatively long total sleep time and relative underestimation of sleep time compared to PSG) and is absent in subjects with objective insomnia (relatively short PSG total sleep time) [40]. The increased amount of beta EEG activity in insomniacs has been conceptually linked to the hyperarousal model of insomnia, e.g., [41], and CRH actions at the locus coeruleus (LC) possibly resulting from an increased CRH activity (see above) have been considered as an explanation for increases in high-frequency EEG activity in insomniacs [17]. Results from the multiple sleep latency test (MSLT) in patients with insomnia have provided further support for the hyperarousal hypothesis of primary insomnia. Contrary to the expectations, patients with insomnia do not fall immediately asleep at daytime when they get the opportunity to take a nap, but they show normal or even prolonged daytime sleep latencies instead [42–44].

1.2.3 Neuroimaging

Further direct evidence of the hyperarousal hypothesis of primary insomnia is obtained from structural and functional neuroimaging studies. Since there exist not only very few studies, but these studies have also been performed in small groups, the promising results partly support the hypothesis of a hyperarousal in primary insomnia that might be linked to an imbalance of excitatory and inhibitory inputs into the central nervous system (see below).

In a positron emission tomography (PET) study [45], (1) a reduction of relative metabolism from waking to non-REM sleep was observed in the bilateral frontal cortex, anterior cingulate cortex, medial prefrontal cortex, left occipitoparietal cortex, posterior cingulate cortex, temporoparietal cortex, and thalamus in healthy subjects; (2) however, in patients with insomnia, a decrease in relative metabolism from waking to non-REM sleep was observed only in the bilateral frontal cortex, right occipitoparietal cortex, and left temporoparietal cortex but not in the thalamus, anterior cingulate cortex, and medial prefrontal cortex; (3) furthermore, compared to healthy subjects, patients with insomnia showed a smaller decline in relative metabolism from waking to non-REM sleep in the ascending reticular activating system (ARAS), hypothalamus, thalamus, insular cortex, amygdala, hippocampus, and in the anterior cingulate and medial prefrontal cortices; (4) compared to healthy subjects, patients with insomnia showed a hypometabolism in the bilateral frontal cortex, the left hemisphere superior, temporal, parietal, and occipital cortices, and in the thalamus, hypothalamus, and brainstem reticular formation during wakefulness; (5) beyond that, it is worth mentioning that in this study, insomniacs did not differ from healthy subjects in PSG. Taken together, the authors concluded that – compared to healthy subjects – in patients with insomnia, an increased cerebral metabolism in non-REM sleep results from a lack of decline in activity in subcortical wake-promoting structures.

The report by Nofzinger et al. [45] has often been invoked as the first direct evidence of hyperarousal in insomnia [27, 46] and has been considered as one of the

key references in neuroimaging research related to insomnia and the hyperarousal hypothesis of primary insomnia. However, the presented PET results from seven insomniacs aged 34.2 years ($SD = 8.9$) have not been replicated or scrutinized in a bigger sample so far. Furthermore, the observed hypometabolism during wakefulness appears to be contradictory to the MSLT results presented above and hence to the concept of a generalized and maintained hyperarousal.

In sharp contrast to the findings from the PET studies [45, 47], a single photon emission computed tomography (SPECT) study showed (1) a decreased regional cerebral blood flow in patients with primary insomnia compared to good sleepers in all of the eight observed regions of interest in the first non-REM sleep cycle including frontal medial cortex, thalamus, occipital cortex, basal ganglia, parietal cortex, frontal lateral cortex, temporal cortex, and pons [48]. (2) Compared to good sleepers, the reduced regional cerebral blood flow was significant for frontal medial, occipital, and parietal cortices, and basal ganglia. (3) In addition, within the group of patients with primary insomnia, a significantly decreased activity was found in the basal ganglia compared to the frontal lateral cortex, frontal medial cortex, thalamus, and occipital and parietal cortices. Therefore, the authors concluded that primary insomnia may be associated with an abnormal central nervous system activity during non-REM sleep which may be linked to basal ganglia dysfunction. Interestingly, as pointed out by Desseilles et al. [49], compared to good sleepers, a decreased activity in the previously mentioned regions was also found in the study performed by Nofzinger et al. [45], but during wakefulness rather than during non-REM sleep.

Cortical hypoactivation during wakefulness, namely of the medial and inferior prefrontal cortical areas, was also discovered in the first and so far only functional magnetic resonance imaging (fMRI) study performed in patients with insomnia [50]. Compared to controls, insomniacs showed less activation in the left medial prefrontal cortex and left inferior frontal gyrus regarding both letter fluency and category fluency as assessed by a letter and category fluency task. In contrast to the previously presented PET and SPECT studies, the authors also examined the effect of nonpharmacological treatment. Letter fluency was restored in two regions of the left inferior frontal gyrus but not in the left medial prefrontal cortex, whereas category fluency activation was partly restored in the left medial prefrontal cortex but not in the left inferior frontal gyrus.

A different neurochemical approach was followed in a recently published magnetic resonance spectroscopy (1H-MRS) study [46]. In patients with primary insomnia, reduced daytime overall average brain GABA (gamma-aminobutyric acid) levels, averaged from basal ganglia, thalamus, and parietal, occipital, and temporal white matter and cortical regions, were observed. Remarkably, GABA levels correlated with both subjective and objective sleep measures. In particular, longer wake time after sleep onset (WASO) in outpatient and inpatient polysomnography was associated with lower GABA levels. As GABA, which is the most important and ubiquitous inhibitory neurotransmitter in the central nervous system, is not only involved in sleep–wake regulation but also in the regulation of other processes that are disturbed in insomnia and support the hypothesis of central

nervous system hyperarousal (e.g., EEG, see above), the finding of reduced GABA levels in patients with primary insomnia is in line with the hyperarousal model of primary insomnia. Moreover, reduced GABA levels have also been observed in major depressive disorder [51], even in recovered subjects [52, 53], which, in addition to the mutual clinical and neuroendocrine disturbances mentioned above, suggests a common underlying pathophysiology in primary insomnia and major depressive disorder.

In addition to alterations in neurotransmitter levels, a morphometric magnetic resonance imaging (MRI) study revealed abnormalities in the structure of the brain in patients with chronic primary insomnia [54, 55]. As cognitive and also affective disturbances can occur in primary insomnia, the study included the dorsolateral prefrontal cortex, the orbitofrontal cortex, the anterior cingulate cortex, amygdala, and hippocampus as regions of interest (ROI), since these regions play a central role in the regulation of cognition and mood. The eight subjects with primary insomnia had smaller hippocampal volumes bilaterally compared to the eight normal sleepers, while none of the other regions showed differences in volume between the two groups. The authors concluded that sleep restriction might have a negative influence on neurogenesis in the hippocampus. Alternatively, increased cortisol levels, which are found in some patients with primary insomnia (see above), might explain the reduced hippocampal volumes [16, 17]. The latter explanation would again be consistent with the hyperarousal model of primary insomnia as increased cortisol levels can be ascribed to increased CRH activity. As reduced hippocampal volumes are also observed in major depressive disorder (for a current review see [56]), the results once more suggest a common underlying pathophysiology of MDD and primary insomnia.

1.3 The Cognitive-Behavioral Model and Cognitive-Behavioral Therapy of Insomnia

Besides alterations in brain functions that can be assessed by means of objective measurements as presented above, insomnia, especially when chronic, is accompanied by a myriad of dysfunctional beliefs and attitudes [57] as well as maladaptive habits, which can be addressed by cognitive-behavioral therapy [58, 59]. Dysfunctional cognitions or beliefs as well as maladaptive habits or safety behaviors contribute to the development and maintenance of insomnia. Insomniacs might worry over sleep loss or ruminate over the expected consequences such as, for instance, daytime residual effects. To prevent further sleep loss, they might take daytime naps to compensate for the believed sleep loss at night, which in turn leads to a reduced sleep pressure at night and hence exacerbates insomnia. Insomniacs might also spend excessive time in bed, thereby possibly performing sleep-incompatible activities such as reading or watching TV.

The relation between insomnia, dysfunctional cognitions, maladaptive habits, consequences, and arousal has been summarized in the microanalytical model, also termed the vicious circle of persistent insomnia [60, 61]. Beside cognitive models of the maintenance of insomnia [62], a behavioral model that also addresses underlying neurocognitive processes and emphasizes the role of hyperarousal [63] has been developed.

Nonpharmacological, cognitive-behavioral therapy of insomnia comprises stimulus control therapy, sleep restriction therapy, relaxation techniques, cognitive interventions, paradoxical intention, and sleep hygiene, e.g., [4]. For example, patients are advised to go to bed only when they feel sleepy and intend to fall asleep. Furthermore, they are recommended, for instance, not to take daytime naps, to reduce alcohol and stimulant (caffeine, nicotine) intake, and to keep a fixed wakeup time regardless of the assumed sleep duration in the previous night.

As proposed by Spielman et al. [64], predisposing factors, precipitating events, and perpetuating attitudes and practices (3p-model) account for the onset and course of insomnia. The contribution of predisposing factors remains constant in the development of insomnia. However, over time, the influence of precipitating events will decrease, while the influence of perpetuating habits and behavior increases, thus maintaining insomnia without any marked reduction of sleep disturbance intensity. Therefore, cognitive-behavioral therapy of insomnia, which is focused on perpetuating factors, is not only expected to be an effective treatment but has indeed proven to be effective in a number of studies, e.g., [65–67].

1.4 Sleep–Wake Regulation with Regard to Insomnia and Pharmacological Treatment

Sleep and wake are regulated by a number of different brain structures, which are interconnected directly or indirectly and form neural networks that are driven by various neurotransmitters, hormones, internal and even external stimuli. While specific brain regions, neural networks, and regulatory feedback systems are each responsible for certain aspects of sleep–wake regulation such as circadian control of sleep or the generation of REM and non-REM sleep, the components of sleep–wake regulation do not act independently from each other but are interlinked at several sites. The different sites of action, their neurotransmitters or hormones, receptors, and connections are illustrated in Fig. 1.

In 1949, Moruzzi and Magoun described the ascending reticular activating system (ARAS) as the wake-promoting system of the brain [68]. As, through the years, the components of the ARAS have been identified and some of them have been found to be placed outside the reticular formation (RF), the term ARAS became less common (cf. [69]). In Fig. 1, the histaminergic tuberomammillary nucleus (TMN), the dopaminergic ventral periaqueductal gray matter (vPAG) and ventral tegmental area (VTA), the serotonergic dorsal raphe nucleus (DR), the

excitatory projections to themselves (positive feedback) and to LC and DR (connections not shown in Fig. 1). In this way, a neural network is constituted that generates REM sleep when activity of LDT/PPT neurons predominates, while non-REM sleep is induced by the dominance of the REM-off neurons' activity (LC/DR) in the so-called reciprocal-interaction model of REM/non-REM oscillation [72, 73]. The ascending wake-promoting system is also the target of pharmacological treatment of insomnia. Histamine H1-receptor antagonists such as doxepin or diphenhydramine counteract histamine from the TMN, and serotonin 5-HT_{2A}-receptor antagonists such as trazodone block serotonin action from the DR.

The ascending wake-promoting system is opposed by the ventrolateral preoptic nucleus (VLPO) and the extended part of the ventrolateral preoptic nucleus (eVLPO), respectively, which can be regarded as the sleep-promoting system of the brain. GABAergic projections from the eVLPO/VLPO to the ascending wake-promoting system, especially to the TMN, the LC, and the DR, promote sleep by inhibition of the wake-promoting system in a switch-like manner, which is why the areas involved and their interaction are referred to as the hypothalamic sleep switch [70, 71]. GABA is in the center of hypothalamic sleep regulation, or rather of sleep promotion. Therefore, besides the above-presented pharmacological strategy of counteracting the action of the wake-promoting system by means of histamine receptor antagonists or serotonin receptor antagonists, another approach is to inhibit the activity of the wake-promoting system by (1) enhancing the effect of GABA (use of positive allosteric modulators (PAMs) at GABA_A receptors: barbiturates, benzodiazepines, and the so-called "Z" drugs (zolpidem, zaleplon, eszopiclone) [74]) or (2) activating the eVLPO/VLPO.

Both wake- and sleep-promoting systems are influenced by the suprachiasmatic nucleus (SCN). The SCN has an intrinsic circadian rhythmicity with a phase duration of about 24 h and 11 min, which is also maintained in constant lighting conditions [75]. Beyond that, the intrinsic circadian rhythm of the SCN is adapted to extrinsic rhythms such as day (light) and night (darkness). This process is called *entrainment* and is mediated by the retinohypothalamic tract (RHT) [76]. In broad daylight, cells in the SCN activate the dorsomedial nucleus of the hypothalamus (DMH), which inhibits VLPO activity by GABAergic projections [71]. The SCN also plays a crucial role in the production and release of melatonin, which is released at night or in the dark from the pineal gland. Melatonin from the pineal gland in turn is an agonist at melatonin MT1 and MT2 receptors of the SCN, thus resetting the SCN. As some subtypes of insomnia might at least partly be caused by a shifted circadian rhythm of arousal, a recent pharmacological treatment approach of insomnia utilizes the melatonin receptors of the SCN to reset the SCN by means of the selective melatonin MT1 and MT2 receptor agonist ramelteon.

Besides the influence of the SCN, the hypothalamic sleep switch is modulated by orexinergic neurons from the lateral hypothalamic area (LHA), the posterior hypothalamus (PH), and the perifornical area of the lateral hypothalamus (PeF) [77]. Orexin directly activates the above-mentioned wake-promoting centers and also the cerebral cortex. Loss of orexinergic neurons is observed in narcolepsy, a disease that goes along with excessive daytime sleepiness and sleep attacks [78]. Inducing

sleep by antagonizing orexin at the orexin OX1 and OX2 receptors with the competitive OX1 and OX2 orexin receptor antagonists ACT-078573 (almorexant) or SB-649868 is another upcoming pharmacological treatment approach for primary insomnia [79].

As stated above, the hypothalamic–pituitary–adrenal (HPA) axis is often disturbed in insomnia. Referring again to the hyperarousal model of insomnia, increased activity of the paraventricular nucleus (PVN) might lead to an increased CRH release, which (1) results in an increased activity of the wake-promoting LC and (2) leads to an enhanced release of adrenocorticotrophic hormone (ACTH) from the pituitary gland and cortisol from the adrenal gland. Thus, overactivity and dysregulation of the HPA axis might contribute to the cause of some types of insomnia. Accordingly, the usefulness of CRH receptor antagonists [17] and also glucocorticoid (GR) and mineralocorticoid (MR) receptor antagonists [8] has been discussed. However, to our best knowledge, CRH, GR, or MR receptor antagonists are currently of less importance in the field of emerging insomnia treatments.

1.5 Conclusions

Insomnia is a clinically heterogeneous disease. In a theoretical review, Roth [41] has summarized evidence of sympathetic nervous system hyperarousal in insomnia including (1) elevated levels of circulating catecholamines, (2) increased basal metabolic rate, (3) increased body temperature, (4) altered heart rate variability and reduced respiratory sinus arrhythmia, as well as (5) elevated beta EEG frequency and cortical activation in the EEG. Besides these findings, indications of a cerebral hyperarousal are obtained from neuroimaging studies, as shown above, and are also compatible with the cognitive-behavioral or psychophysiological model of insomnia. As outlined above, the hyperarousal model of insomnia is also in line with a possible common underlying pathophysiology of insomnia and major depressive disorder. Both pharmacological and nonpharmacological therapies are effective in the treatment of primary insomnia, whereby the pharmacological first-line treatment consists of the use of the so-called Z drugs to induce sleep onset and sleep maintenance. Current and emerging pharmacological approaches are mainly based on H₁ histamine receptor antagonism, 5-HT₂ serotonin receptor antagonism, GABA_A receptor agonism, MT₁ and MT₂ melatonin receptor agonism, and OX₁ and OX₂ orexin receptor antagonism (for a comprehensive review of current and investigational approaches in treating insomnia, see [80], for emerging anti-insomnia drugs, see [79]). Besides the development of more selective drugs, future research is needed to identify subtypes and biomarkers of insomnia in order to choose a personalized treatment with the best possible efficacy and the lowest risk of side effects.

2 Hypersomnias of Central Origin: Narcolepsy and Other Forms of Hypersomnia

Excessive daytime sleepiness (EDS), the main symptom of all hypersomnias, constitutes a severe impairment of everyday life. In our fast-paced society, continued alertness is essential for almost all areas of function, especially technologically assisted but often monotonous activities such as driving. The definition of EDS comprises the inability to stay awake and alert during the major waking episodes of the day. The severity of EDS is variable, and in severe cases, it may lead to imperative sleep attacks. EDS is a common feature of all hypersomnias and may be associated with an increased total daily amount of sleep (without the feeling of restoration), i.e., hypersomnia with long sleep time, which can be assessed by means of actigraphy or polysomnography. Other forms of hypersomnia may show no relevant increase in total sleep time, but an incapability of maintaining wakefulness during the day, which can be quantified by the multiple sleep latency test (MSLT). The hypersomnias defined by the ICSD-2 [2] are shown in Table 4.

2.1 Key Features

2.1.1 Narcolepsy

Narcolepsy is a disabling and life-long sleep–wake disorder characterized primarily by EDS, which appears usually as the first symptom. Patients with narcolepsy typically feel refreshed after short naps, but usually, after a few hours, they feel sleepy again. If sleepiness is severe, unpredictable and irresistible sleep attacks as well as automatic behavior may appear in routine situations. Cataplexies are

Table 4 Hypersomnias defined by the ICSD-2 [2]

1	Narcolepsy with cataplexy
2	Narcolepsy without cataplexy
3	Narcolepsy due to medical condition
4	Narcolepsy, unspecified
5	Recurrent hypersomnia
	Kleine–Levin syndrome
	Menstrual-related hypersomnia
6	Idiopathic hypersomnia with long sleep time
7	Idiopathic hypersomnia without long sleep time
8	Behaviorally induced insufficient sleep syndrome
9	Hypersomnia due to medical condition
10	Hypersomnia due to drug or substance
11	Hypersomnia not due to substance or known physiological condition (nonorganic hypersomnia)
12	Physiological (organic) hypersomnia, unspecified (organic hypersomnia)

pathognomonic for narcolepsy and characterized by a sudden bilateral loss of muscle tone triggered by strong emotions such as laughter or surprise. Cataplexy can be localized or include all muscle groups. The recovery is immediate and complete after some seconds up to a few minutes. Associated features such as hypnagogic hallucinations (vivid perceptual experiences typically occurring during sleep onset or awakening) or sleep paralysis (inability to move or speak during the transition between sleep and wakefulness) are experienced by 40–80% of patients suffering from narcolepsy with cataplexy. In addition, nocturnal sleep disruption is a common but unspecific feature in patients with narcolepsy. The diagnostic criteria for narcolepsy with cataplexy are listed in Table 5.

In narcolepsy without cataplexy, the refreshing character of naps is pathognomonic (Table 6). In this form of narcolepsy, a nocturnal polysomnography followed by a pathological MSLT is mandatory to confirm the diagnosis.

A short sleep latency of less than 10 min and sleep onset REM periods (SOREMP) are frequent findings in narcolepsy. In the MSLT, a mean sleep latency of less than or equal to 8 min and two or more SOREMPs are very specific findings and required for the diagnosis of narcolepsy without cataplexy [81].

Typically, hypocretin-1 levels in the cerebrospinal fluid (CSF) are less than or equal to 110 pg/ml [82]. However, up to 10% of patients with cataplexy have been

Table 5 Diagnostic criteria for narcolepsy with cataplexy [2]

-
- 1 The patient has a complaint of excessive daytime sleepiness occurring almost daily for at least 3 months
 - 2 A definite history of cataplexy, defined as sudden and transient episodes of loss of muscle tone triggered by emotions, is present. (To be labeled as cataplexy, these episodes must be triggered by strong emotions – most reliably laughing or joking – and must be generally bilateral and brief. Consciousness is preserved, at least at the beginning of the episode.)
 - 3 The diagnosis of narcolepsy with cataplexy should, whenever possible, be confirmed by nocturnal polysomnography followed by an MSLT; the mean sleep latency on MSLT is less than or equal to 8 min and two or more SOREMPs are observed following sufficient nocturnal sleep (minimum 6 h) during the night prior to the test. Alternatively, hypocretin-1 levels in the CSF are less than or equal to 110 pg/ml or one third of mean control values
 - 4 The hypersomnia is not better explained by another sleep disorder, medical or neurological disorder, medication use, or substance use disorder
-

Table 6 Diagnostic criteria for narcolepsy without cataplexy [2]

-
- 1 The patient has a complaint of excessive daytime sleepiness occurring almost daily for at least 3 months
 - 2 Typical cataplexy is not present, although doubtful or atypical cataplexy-like episodes may be reported
 - 3 The diagnosis of narcolepsy without cataplexy must be confirmed by nocturnal polysomnography followed by an MSLT; the mean sleep latency on MSLT is less than or equal to 8 min and two or more SOREMPs are observed following sufficient nocturnal sleep (minimum 6 h) during the night prior to the test
 - 4 The hypersomnia is not better explained by another sleep disorder, medical or neurological disorder, medication use, or substance use disorder
-

reported to have normal hypocretin-1 levels in the CSF. At a genetic level, narcolepsy is very tightly associated with the human leukocyte antigen (HLA) DQB1*0602. However, this is no exclusive feature of narcolepsy, as approximately 25% of the general population is positive for the same antigen. Negative HLA typing may be useful to exclude the diagnosis in selected cases.

Narcolepsy is a rare disease with a prevalence of about 0.05% with specific ranges between 0.002% in Israel and 0.18% in Japan [83–85]. Although the disease may start at any age, there exist two peaks: one between the first and second decade of life, the other between the third and fourth decade of life.

2.1.2 Other Forms of Hypersomnia

Idiopathic hypersomnia is characterized by constant and severe EDS with prolonged and unrefreshing naps of up to several hours. There are two forms: with and without prolonged total sleep time. Due to diagnostic uncertainty, demographics and epidemiology are principally unknown. Usually, the onset of idiopathic hypersomnia occurs before the age of 25 years.

Recurrent hypersomnia including the Kleine–Levin syndrome and menstrual-related hypersomnia is characterized by the periodical reappearance of excessive sleepiness. These episodes with a duration of 2 days to 4 weeks recur at least once a year. Cases with episodes related to the menstruation cycle are rarely described and must be differentiated from the premenstrual syndrome. In the Kleine–Levin syndrome, the recurrent episodes of hypersomnia are associated with behavioral abnormalities such as binge eating, hypersexuality, and abnormal behavior, which are no longer present between the episodes.

2.2 *Pathophysiological Concepts of Narcolepsy*

2.2.1 Changes in the Orexin/Hypocretin System

In the last decades, several studies have shown the pivotal role of the neuropeptide orexin/hypocretin in the pathophysiology of narcolepsy [86–89]. Orexins/hypocretins are excitatory neuropeptides produced in the lateral and posterior hypothalamus. Projections of orexin/hypocretin neurons are widely distributed in the brain. Mice and dogs with a defect in the orexin/hypocretin gene show phenotypes with similar symptoms as in human narcolepsy [90–94]. Correspondingly, a reduction of orexin A levels in the cerebrospinal fluid of approximately 90% of patients with narcolepsy [82, 95] as well as a decreased number of orexin/hypocretin-containing neurons in the hypothalamus in postmortem brain tissue [78] have been shown. Despite the genetic link in animal models, no link with the orexin/hypocretin gene could be found in most patients. Only one child with an early onset of narcoleptic symptoms showed a genetic polymorphism in the orexin/hypocretin gene [96]. This

leads to the conclusion that the orexin/hypocretin deficiency is rather acquired than congenital. An immune-mediated degeneration of orexin neurons is suggested because of the strong association with the HLA-system in narcolepsy [97, 98].

Because of the strong relationship between orexin and the sleep–wake system and due to the orexin deficiency in narcoleptic patients, the development of orexin receptor agonists is a desirable therapeutic option. In narcoleptic mice, cataplexies were inhibited and wakefulness was maintained after the acute administration of orexin A [99]. Apart from therapeutical possibilities, measuring orexin A in the CSF represents a valuable diagnostic test.

2.2.2 HLA Association and Immunological Aspects

The human leukocyte antigens (HLA) are all-surface glycoproteins genetically coded on chromosome 6, supporting the immune system in the recognition of foreign antigens. HLA are surface proteins on cells and genetically coded on chromosome 6. The relationship between narcolepsy and the HLA-system is one of the strongest in comparison to other diseases such as multiple sclerosis. But, it is still unclear if an autoimmune reaction is the origin of the disease. In 1984, Juji et al. found that all narcoleptic patients in their study in Japan were positive for HLA DR2 [97]. Similar relationships were later reported in Europe and North America. The association is quite unspecific as 12–38% of the human population is HLA-DR2-positive. In addition, some cases of narcoleptics who are HLA-DR2-negative are reported, especially with a background of familial clustering.

The HLA-DQB1*0602 subtype is most specific: Almost 90% of all narcoleptic patients with cataplexy have the HLA-DQB1*0602 haplotype as a common trait as compared to approximately 30% of the normal population. Notwithstanding this association, some studies showed DR2-positive monozygotic twins with a discordance for hypersomnia, REM sleep latency, and cataplexy [100]. These results strongly favor a multifactorial pathogenetic model of narcolepsy. In accordance with the autoimmune hypothesis of narcolepsy, increased tumor necrosis factor (TNF)- α levels were found in narcoleptic patients [101, 102]. However, other studies did not confirm these results [103, 104]. The TNF- α gene is located in the HLA III region, and TNF influences the peripheral immune system through the lymphatic pathway [105]. TNF- α promoter polymorphisms have been found in narcoleptic patients in European countries [106] and in the Japanese population [107]. In addition, elevated soluble TNF-R p75 levels were found in narcoleptic patients in comparison to healthy controls [104]. In the light of these results, an autoimmune process involving altered TNF signaling seems plausible but has not been proved yet. It has been found that Interleukin-6 levels are increased in narcoleptic patients [101]. Interestingly, a rare form of HLA-DR2-negative narcolepsy has also been shown to be associated with an α -interferon gene polymorphism. On a genetic basis, obligatory but still unknown environmental factors are thought to cause structural or functional changes in the CNS causing the symptoms of narcolepsy.

2.2.3 Disturbed Sleep–Wake Regulation

Symptoms such as cataplexy and sleep paralysis (muscle atonia) associated with hypnagogic hallucinations may be interpreted as an intrusion of REM sleep into wakefulness. This is underlined by the frequent finding of a reduced REM sleep latency or SOREMPs in narcoleptic patients [108]. A model of narcolepsy that supposes an increased REM sleep pressure on the basis of a cholinergic hyperactivity associated with a dopaminergic hypoactivity in sleep-regulating centers of the brain stem has been postulated. In addition, a reduced norepinephrinergic activity has been shown in the locus coeruleus [108]. The instability of the sleep–wake regulation may be due to the lack of orexin and its important role in stabilizing the waking part of this system [109].

2.2.4 Metabolic Aspects of Narcolepsy

In early reports, it stands out that narcolepsy is associated with obesity [110, 111]. Recent studies confirmed the elevated obesity levels reported in narcoleptics [87, 112–116]. Interestingly, the change of body weight often coincides with disease onset [117]. In the light of the known increased rate of obesity, patients with narcolepsy may be more prone to cardiovascular risk factors than the general population. However, HLA-DQB1*0602, which is highly associated with narcolepsy, is protective for type 1 diabetes mellitus [118]. One study suggested that narcolepsy is associated with an increased incidence of type 2 diabetes mellitus. On a group level, this was independent of the body weight [119]. Whereas no definite information is available about food intake in narcoleptic patients, glucose intake specifically induces sleepiness in these patients as compared to healthy controls [120]. Various reasons such as pharmacological treatment or eating behavior might play a role in weight change. Despite the well-known obesity-inducing effect of many antidepressants, no obvious differences in the prevalence of obesity between medicated and drug-naïve patients were apparent in a study by Schuld et al. [121]. Obesity in narcolepsy may be related to additional endocrine abnormalities such as a putative peripheral leptin deficiency in narcoleptic patients [122–124].

2.2.5 GABA

An important role of the GABAergic system in the pathophysiology of narcolepsy is suggested by the global impact of GHB (gamma-hydroxybutyrate), a metabolite of GABA and an agonist of its receptor, on all three main symptoms of narcolepsy: fragmented night sleep, cataplexy, and hypersomnia.

As a metabolite of GABA, GHB promotes slow-wave sleep and improves nocturnal sleep disturbances in narcolepsy [125]. The binding of GHB to the GABA_B receptors may be responsible for its sleep-promoting effect. The sites of action of GHB, an endogenous fatty acid, are the GHB receptor and the GABA_B

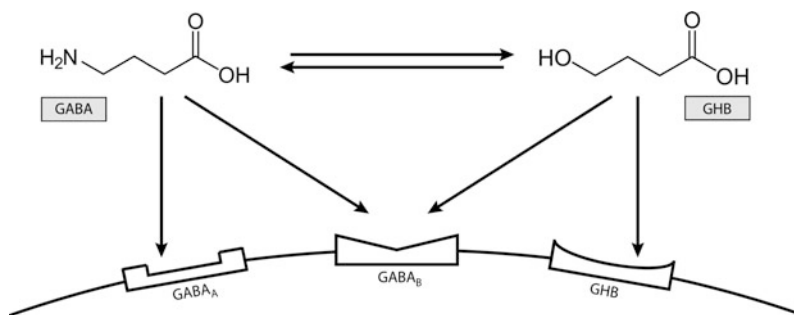


Fig. 2 GABA, GHB, and their receptors. Possible pharmacological mechanisms of GHB: (a) activation of GHB receptors, (b) activation of GABA_B receptors, or (c) metabolism to form GABA, which binds to GABA_A and GABA_B receptors

receptor [126–128], the latter being the more important receptor. As the precursor of GHB, GABA exerts its effects via GABA_A, GABA_C, and GABA_B receptors [129, 130]. The receptors playing a role in the pharmacological treatment of narcolepsy are presented in Fig. 2. Recent studies showed that orexin/hypocretin neurons receive, among others, input from GABAergic neurons in the preoptic area [131].

Narcolepsy is characterized by an inadequate occurrence of REM sleep-associated behavior. Interestingly, each side of the proposed flip-flop switch for the REM sleep control is heavily innervated by GABAergic neurons [132]. Moreover, one study showed that there may be a susceptibility locus for narcolepsy on chromosome 4. One of the genes on this chromosome, which may be related to narcolepsy, is the GABA receptor gene beta-1 [133]. The orexin/hypocretin system integrates, among others, input from GABA [134]. The orexin/hypocretin system plays a pivotal role in the sleep–wake system, energy homeostasis, and many autonomic and neuroendocrine circuits [135, 136]. GABA reduces the activity of orexinergic/hypocretinergic cells [137, 138]. An important role of the GABAergic system in the pathophysiology of narcolepsy is suggested by the effective treatment of cataplexy, hypersomnia, and perturbed sleep using GHB, a metabolite of GABA and an agonist of its receptor.

2.3 Therapeutic Aspects

2.3.1 Treatment of Hypersomnia

The treatment of hypersomnia has to be adapted to the individual needs of the patient. Centrally acting wakefulness-promoting agents such as amphetamines and their derivatives acting on dopaminergic systems of the midbrain increase monoaminergic transmission (catecholamine but also serotonin) through multiple

mechanisms. The efficiency of D-amphetamine and metamphetamine in the treatment of EDS has been shown by numerous studies [139–141].

According to European guidelines, modafinil is a first-line treatment for excessive daytime sleepiness [142]. It is structurally unrelated to amphetamines, has no sympathetic side effects, and has only a low potential for abuse. Although the precise mechanism of action is still in debate, it has been considered that modafinil interacts with adrenergic [143], dopaminergic [144–146], and serotonergic/GABAergic systems [145].

2.3.2 Treatment of Cataplexy, Hypnagogic Hallucinations, Sleep Paralysis

The anticataplectic effect of tricyclic antidepressants (TCAs) has already been shown in 1960 [147]. Whether this effect is due to the TCA's ability to increase the muscle tone [148] or due to a suppression of REM sleep is not known. Although clomipramine is the most widely used TCA in narcolepsy, an anticataplectic effect has also been shown for desmethylinipramine, protriptyline, imipramine, and desipramine [149]. Selective serotonin reuptake inhibitors (SSRIs) also strongly suppress nocturnal REM sleep and have an anticataplectic effect [150–153]. The most commonly used SSRI is fluoxetine, but several others such as fluvoxamine, zimeldine, femoxetine, and paroxetine are used as well. Venlafaxine, an antidepressant blocking the reuptake of norepinephrine and serotonin, and other antidepressants selectively blocking the norepinephrine reuptake, such as viloxazine, reboxetine, and atomoxetine, are also effective in the therapy of narcolepsy [150, 154, 155].

Beyond these substances, a few other compounds are known to have anticataplectic effects. Mazindol [139, 156, 157], phenelzine [158], and selegiline [159, 160] may improve cataplexies but the data is sparse. Currently, sodium oxybate (see below) is recommended as a first-line treatment for cataplexies because of its additional effects on daytime sleepiness, hypnagogic hallucinations, sleep paralysis, and disrupted night sleep [142, 161].

2.3.3 Treatment of Disrupted Nocturnal Sleep Due to Narcolepsy

In severe cases with predominant fragmented night sleep, hypnotics such as short-acting benzodiazepines may be an effective treatment apart from sodium oxybate. One study with triazolam revealed an improvement in sleep efficiency and sleep quality but not in daytime sleepiness [162]. Because of the lack of data regarding long-term administration and the well-known risk of abuse, other treatment possibilities such as sodium oxybate should be preferred. Modafinil may increase sleep efficiency [163], but the most advisable treatment of disrupted sleep is sodium oxybate (see below).

Sodium Oxybate

It was as early as 1979 when Broughton and Mamelak suggested that sodium oxybate, the sodium salt of GHB, has beneficial effects on narcoleptic symptoms [164]. Daytime alertness and night sleep were improved and the number of cataplexies was reduced [164]. Interestingly, GHB was shown to decrease the latency to slow-wave sleep in healthy subjects [165] but not in narcoleptic patients [166]. GHB may induce REM sleep but does not increase its duration. A decreased REM sleep latency is reported in patients with insomnia, depression [167], and narcolepsy [166, 168, 169] but not in patients with idiopathic EDS [170]. GHB in doses of up to 9 g/day significantly reduces the frequency of cataplectic attacks [171, 172]. This effect usually starts after a latency of several weeks. The underlying mechanism of the REM-inducing effect is still unknown, but dopaminergic mechanisms may be involved [165]. Apart from the strong antcataplectic effect, GHB also ameliorates excessive daytime sleepiness, disturbed night sleep, hypnagogic hallucinations, and sleep paralysis [173, 174].

3 Pathophysiology of Sleep-Related Movement Disorders: Restless Legs Syndrome and Periodic Limb Movement Disorder

In the second edition of the International Classification of Sleep Disorders (ICSD-2), the restless legs syndrome (RLS) and periodic limb movement disorder (PLMD) are classified as “sleep-related movement disorders” [2]. These disorders are defined as conditions that are primarily characterized by relatively simple, usually stereotyped, movements. In addition, complaints about disturbed sleep, daytime sleepiness, or fatigue are mandatory for the diagnosis. Although not involving stereotyped movements per se, RLS has been included into this diagnostic category due to its close association with periodic limb movements (PLM).

3.1 Key Features

RLS is a disorder characterized by uncomfortable dysesthesias or paresthesias in the legs and less frequently in the arms, occurring primarily at rest in the evening hours. Typically, the symptoms are alleviated by movement of the involved limbs. As a consequence, patients suffer from insomnia leading to an impaired quality of life and mental health. In 1995, the International RLS Study Group developed standardized criteria for the diagnosis of RLS, which have been recently modified [175] and included into the ICSD-2 criteria. Four criteria are essential to establish the diagnosis of RLS; supportive and associated clinical features are given in Table 7.

Table 7 Criteria for RLS [175]

Essential diagnostic criteria for RLS	
1	An urge to move the legs, usually accompanied or caused by uncomfortable and unpleasant sensations in the legs. (Sometimes the urge to move is present without the uncomfortable sensations and sometimes the arms or other body parts are involved in addition to the legs)
2	The urge to move or unpleasant sensations begin or worsen during periods of rest or inactivity such as lying or sitting
3	The urge to move or unpleasant sensations are partially or totally relieved by movement, such as walking or stretching, at least as long as the activity continues
4	The urge to move or unpleasant sensations are worse in the evening or night than during the day or only occur in the evening or night. (When symptoms are very severe, the worsening at night may not be noticeable but must have been previously present)
Supportive clinical features	
Family history	The prevalence of RLS among first-degree relatives of people with RLS is 3–5 times greater than in people without RLS
Response to dopaminergic therapy	Nearly all people with RLS show at least an initial positive therapeutic response to either levodopa or a dopamine-receptor agonist at doses considered to be very low in relation to the traditional doses of these medications used for the treatment of Parkinson's disease. This initial response is not, however, universally maintained
Periodic limb movements	Periodic limb movements in sleep (PLMS) occur in at least 85% of people with RLS; however, PLMS also commonly occur in other disorders and in the elderly. In children, PLMS are much less common than in adults
Associated features	
Natural clinical course	The clinical course of the disorder varies considerably, but certain patterns have been identified that may be helpful to the experienced clinician. When the age of onset of RLS symptoms is less than 50 years, the onset is often more insidious; when the age of onset is greater than 50 years, the symptoms often occur more abruptly and more severely. In some patients, RLS can be intermittent and may spontaneously remit for many years
Sleep disturbance	Disturbed sleep is a common major morbidity for RLS and deserves special consideration in planning treatment. This morbidity is often the primary reason the patient seeks medical attention
Medical evaluation/physical examination	The physical examination is generally normal and does not contribute to the diagnosis except for those conditions that may be comorbid or secondary causes of RLS. Iron status, in particular, should be evaluated because decreased iron stores are a significant potential risk factor that can be treated. The presence of peripheral neuropathy and radiculopathy should also be determined because these conditions have a possible, although uncertain, association and may require different treatment

Polysomnographic studies have shown that most patients with RLS show PLM during sleep (PLMS) and relaxed wakefulness (PLMW). Population-based surveys using standardized diagnostic criteria have shown that 5–10% of the general population in Europe and North America exhibits the corresponding cardinal symptoms, identifying RLS as one of the most common neurological or sleep-related movement disorders [176]. The prevalence of RLS increases with age, and females are twice as likely to be affected. Although a definite age of onset cannot be determined, it has been reported that in approximately 40% of the adults with RLS, the onset of symptoms course prior to the age of 20 years. The onset of restless legs symptoms often follows a fluctuating course with periods of improvement or remission over several weeks or months, and rarely even years. The expression of symptoms often varies among patients. Overall, however, the frequency and severity of symptoms tend to progress with age, and permanent remissions are rare.

PLM are highly regular, jerky, stereotyped, nonepileptic movements mostly of the lower extremities characterized by involuntary repetitive extensions of the big toe, often accompanied by flexions of the hip, knees, and ankles. In some cases, the arms may also be affected. In the early 1980s, Coleman [177] was the first to propose standardized scoring criteria for PLMS that were modified by the American Sleep Disorders Association (ASDA) in 1993 [178] and codified in the ICSD-2 [2]. Recently (2007), new standards for recording and scoring PLM were proposed by the World Association of Sleep Medicine (WASM) [179]. PLM, clinical sleep disturbances, and daytime sleepiness or fatigue, which cannot be accounted for by another primary sleep disorder, are the essential features of PLMD. However, PLMS are found in a large variety of sleep disorders including insomnia, sleep apnea, narcolepsy, Parkinson syndrome, and REM sleep behavior disorder, but they occur most frequently in patients with RLS. Although PLMS are common, pure PLMD is thought to be rare. The clinical significance of PLMS in the absence of RLS or pure PLMD is a controversial issue as PLMS constitute a common finding even in the absence of complaints about sleep disruption, particularly in the elderly. PLMS may be associated with an EEG arousal or a brief awakening during nocturnal polysomnography, but patients are often unaware of the limb movements or even the sleep disruptions, and it is the observation of the bed partner that suggests the presence of PLMS. However, there is growing evidence that PLMS are associated with a significant sleep state instability and might constitute a cardiovascular risk factor with a potential long-term negative outcome [180] (Fig. 3).

3.2 Pathophysiological Concepts

Most authors agree that both conditions have their origin in the central nervous system, and various locations including subcortical regions, the brainstem, and the spinal cord are favored as the primary anatomic locus. Pharmacological, neuroimaging, and electrophysiological studies suggest a dysfunction of the supraspinal



Fig. 3 Polysomnography in restless legs syndrome including periodic leg movements. 90-s epoch of a nocturnal polysomnography containing seven periodic leg movements during sleep (PLMS). The PLMS (in this epoch only present in the right leg) occurs every 10–15 s during sleep stage 2. Each leg movement is associated with an EEG arousal. *From top to bottom:* two electrooculography (EOG) channels, three electroencephalography (EEG) channels, one chin electromyography (EMG) channel, electrocardiography (ECG), eight EEG channels, airflow, respiratory movements of the thorax and abdomen, snoring sound recording, right and left leg EMG of the tibialis anterior muscle, and right and left EMG of the extensor digitorum muscle

inhibitory system triggering RLS and PLM. Whether pure PLMD represents a singular disease entity or is part of the phenotypical spectrum of RLS has not been conclusively elucidated.

Previous studies suggest a strong genetic contribution to idiopathic RLS, with heredity estimates of about 50%. Linkage and family-based approaches suggest a complex genetic disorder. Recent genome-wide association studies identified genetic risk variants playing a role in early embryonic stages, each with genome-wide significance within four genomic regions (*MEIS1*, *BTBD9*, *MAP2K5/LBXCOR1*, *PTPRD*) [181]. *MEIS1* and *LBXCOR1* have a so far unexplored function in the adult central nervous system. *MEIS1* encodes a homeodomain transcription factor involved in multiple developmental processes in vertebrates. *LBXCOR1* is a corepressor of *LBX1*, which in turn is selectively expressed in a subset of dorsal horn interneurons during embryonic development of the spinal cord. The *LBX1/LBXCOR1* pathway is indispensable for the generation of a GABAergic phenotype in these interneurons and possibly involved in the modulation of pain and sensory information processing. However, the exact function of this gene is not known

either. *BTBD9* encodes a BTB-POZ domain and has also been found to be associated with PLMS in patients with RLS [182]. *PTPRD* encodes a protein-tyrosin-phosphatase type delta and is involved in axonal guidance and the termination of spinal motor neurons during embryonic development. The identification of developmental factors for RLS questions previous pathophysiological concepts. Based on the immediate and striking efficacy of dopaminergic and opioidergic drugs and the triggering of RLS following treatment with dopamine receptor blocking agents, there is evidence of the involvement of the dopaminergic/opioidergic system in the pathogenesis of this disorder. However, despite what is known about the genetic background and pharmacological challenges, the etiology of RLS still remains unclear (for a comprehensive review see [183]). RLS can be secondary to other disorders or conditions. The main causes include pregnancy, especially in the later trimesters with complete loss of symptoms after delivery, iron deficiency, and end-stage renal disease. About 20–30% of uremic patients meet the clinical criteria for the diagnosis of RLS. Iron deficiency may also be a causative factor for the development of RLS. In addition, several other medical and neurologic conditions have been associated with RLS including neuropathy, radiculopathy, and rheumatoid arthritis.

For PLM, there is strong evidence of a sleep state-dependent increased excitability or loss of inhibitory influences at various levels of the motor pathway including the motor cortex and other supraspinal structures. It seems that the spinal cord provides everything necessary to generate and maintain a rhythmic motor activity of the lower limbs. During sleep, inhibitory influences on spinal processes are dampened – as evidenced by the induction of the Babinski reflex during NREM sleep in healthy subjects – and it might be only a small step to further reduce these influences below a critical level. Spinal pathological processes but also disorders of subcortical structures at the level of the brainstem or basal ganglia are especially likely to give rise to PLMD. While the rhythmicity can be generated within the spinal cord, the temporal association with other physiological processes suggests the brainstem – at the level of the reticular substance – as the “coordinating” center in the case of an intact spinal cord. Given the high prevalence of PLM in the general population, increasing with age, and the systematic coupling with the cyclic alternating A phase during sleep, it could also be argued that PLM are physiological events occurring during all forms of stable sleep – but maybe only in persons with a certain but by no means rare predisposition. This, in turn, would mean that the disturbed sleep in RLS induces the PLM rather than vice versa. The corresponding sensory side of RLS is less well understood. While it seems that peripheral processes are able to trigger RLS in selected subjects, the preponderance of evidence indicates abnormalities in sensory processing rather than sensory input. An as yet concealed process possibly involving hypersensitization is prompted by another process yet to be defined that is closely bound to the circadian system and for which melatonin could be a prime candidate. However, iron metabolism and dopamine also show characteristic variations over the 24-h day, with evidence suggesting that the amplitude of these variations is larger in RLS subjects. Most

likely diverse factors such as iron and dopamine act in concert to produce restless legs symptoms [184].

Although several conditions have been associated with RLS, for many of them, it is unknown how they relate to the onset and course of RLS. This applies not only to the association of RLS with peripheral neuropathy but also to the numerous pathophysiological findings such as impaired temperature perception, increased pain threshold, reduced intracortical inhibition, increased spinal hyperexcitability, decreased brain iron content, or possible thalamic structural or metabolic alterations. On the other hand, end-stage renal dysfunction and pregnancy are conditions that precede the occurrence of RLS. RLS is a disorder with many faces, and prospective studies are needed to determine what is a symptom, a consequence, or a causally operating factor of this disorder [184].

3.3 *Therapeutic Aspects*

The goal of treatment for RLS is to eliminate or minimize symptoms, thus improving overall quality of life and daytime functioning. The decision to start treatment is an individual one depending on the severity and frequency of symptoms and the extent to which symptoms interfere with daily activities. Since a causal therapy is only possible for secondary forms, the first step is to identify any primary etiologies. Therefore, a careful history should be taken to check whether the patient suffers from renal diseases or iron depletion or has taken any substances known to trigger or worsen RLS. Studies have shown that iron-replacement therapy improves the symptoms. Magnesium supplementation may have beneficial effects in mildly affected RLS patients. Benefit of folic acid has been shown in patients with folic acid deficiency and in pregnant women. RLS secondary to uremia can be successfully treated by renal transplantation. Nonpharmacological treatment primarily consists of physical measures such as moving the legs and sensory stimuli or modifications of lifestyle including avoidance of alcohol, nicotine, or caffeine. If conservative measures and treatment of secondary causes do not adequately control the symptoms, pharmacological agents may be considered. Treatment of RLS is a rapidly developing field, and it is only for a few years that large-scale, multicenter trials have been published. A number of patient groups such as pregnant women, children, and patients with additional psychiatric, neurological, or other disorders warrant special attention because of concerns that may complicate the management of their symptoms [185, 186].

Therapy of PLMD, if indicated, is assumed to be similar to the treatment of RLS, although there are insufficient treatment trials targeting PLMD (for review see [187]). However, isolated PLMS generally do not cause insomnia and are not associated with daytime sleepiness or abnormalities in sleep latency tests. Therefore, it is not clear whether PLMD requires treatment unless it worsens sleep.

3.3.1 Dopaminergic Agents

Levodopa

Evidence-based guidelines have identified levodopa as effective in the treatment of RLS [187]. Levodopa plus a decarboxylase inhibitor (carbidopa or benserazide) generally result in a robust initial relief after the first dose. Controlled studies have shown the efficacy of levodopa in idiopathic and uremic RLS. Levodopa is recommended for patients with intermittent but not daily RLS. It is also useful as a diagnostic probe for ascertaining the responsiveness to dopaminergic drugs. Regarding the treatment of PLMD, no controlled study has been reported so far.

Dopamine Agonists

Dopamine agonists are regarded as a first-line treatment for moderate to severe primary RLS needing daily treatment. This is due to their well-documented effectiveness and overall good tolerability (for review see [188]). Dopamine agonists differ with respect to pharmacokinetics (e.g., half-life), dopamine receptor profiles, potential serious side effects, availability of long-term experience, and licensing status. There is a need to increase the dosage slowly, making these drugs less suitable to use on an as-needed-basis. Ropinirole and pramipexole have been approved both in the EU and US for the treatment of adult patients with moderate to severe idiopathic RLS. Several placebo-controlled and open-label studies have shown that ropinirole and pramipexole are effective in significantly improving both subjective and objective measures of RLS for time spans between one night and several months [189, 190]. Rotigotine is designed to be administered as a transdermal patch for 24-h continuous dopaminergic stimulation. A recent multicenter controlled study has shown that rotigotine significantly relieved the night- and daytime symptoms of idiopathic RLS [191].

3.3.2 Opioids

Opioids such as tramadol and tilidine are generally considered as a second-line treatment for RLS. They are recommended if the symptoms do not respond to dopaminergic medication or in the case of nontolerance or augmentation when using these agents. Compared to dopaminergic drugs, the evidence of opioids in the treatment of RLS is sparse; however, most experts find that opioids can be useful, without much risk for addiction, although patients must be monitored for the development of a dependence [185, 186].

3.3.3 Gabapentin and Other Anticonvulsants

In patients with painful sensory symptoms, gabapentin may be considered as a first-line treatment. Although, in idiopathic RLS, subjective relief of RLS symptoms could be demonstrated with valproic acid, the number of PLM did not change [192].

3.3.4 Benzodiazepines

In the past, benzodiazepines were first-line treatments for patients with RLS, but were replaced by more effective drugs. The results of treatment studies are not consistent and a long-term efficacy of benzodiazepines has not been reported so far. However, clonazepam at a dose of 0.5–2 mg/day before bedtime has proven effective in treating both sensory and motor symptoms and improve sleep quality. Currently, it is regarded as an alternative treatment strategy of second choice in RLS and PLMD. Particularly in patients with RLS- and PLMD-associated poor sleep, zopiclone, zolpidem, or zaleplon may be helpful for insomnia when used intermittently [185].

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Insomnia: Differential Diagnosis and Current Treatment Approach

J.F. Pagel and Gerald Kram

Abstract Insomnia is a common complaint that chronically affects at least 14% of the population, leading to deficits in waking performance and quality of life. Insomnia can occur as a primary condition, but it can also be secondary to a wide spectrum of medical, psychiatric, and sleep disorders. The principal treatment strategies for insomnia include addressing comorbid conditions, cognitive and behavioral therapies, and the utilization of hypnotic medications when appropriate, ideally ones with limited potential for addiction or overdose, and low toxicity. It is remarkable that most drugs affecting the global state of sleep exert their effects through one primary neurotransmitter receptor system – the GABA receptor. When utilized as hypnotics, agents with general and indiscriminate effects on the GABA receptor can induce a combination of sedative, anxiolytic, amnestic, and even anesthetic effects, often with negative and detrimental consequences for the patient. However, recently developed hypnotics, exerting primary effects at the $\alpha 1$ subtype GABA-A receptor system, have been shown to have long-term efficacy as well as low toxicity, low addictive and overdose potential.

1 Insomnia: Current Diagnostic Differential and Approach to Treatment

About seventy five million adults (40% of the American population) report that they occasionally experience insomnia, while twenty five million (11–14%) report having an ongoing problem with chronic insomnia [1, 2]. There is a strong association between insomnia and other disease states, with many conditions having insomnia as a major symptom. As a consequence, insomnia is generally divided

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into either primary insomnia, i.e., that which has no identifiable antecedent cause, or insomnia secondary to other types of sleep problems or underlying medical or psychiatric illness [3]. Almost all chronic diseases result in physical or mental discomfort for the patient, inducing disturbances in the state of sleep. If the insomnia is secondary to underlying medical, psychiatric, or sleep-associated disease process, initial evaluation and treatment is most often directed towards that underlying disease. Whatever the cause, the complaint of insomnia is defined as difficulty with sleep initiation, duration, consolidation, or restorative quality, as well as associated impairment in daytime functioning [3]. The daytime functional impairment for patients with insomnia can be fatigue, impaired memory or concentration, mood disturbance, daytime sleepiness, reduced motivation or energy, tension, headaches or gastrointestinal symptoms, as well as concerns and worries about their difficulties with sleeping.

2 The Primary Insomnias Diagnosis and Treatment

The primary insomnias include psychophysiological insomnia, a form of conditioned insomnia based on principles of stimulus–response; paradoxical insomnia, a form of insomnia in which the subjective complaint is much worse than objectively testable parameters; and idiopathic insomnia, a form of insomnia developing in childhood with likely genetic predisposition. The symptoms of all of these insomnias have been shown to improve with cognitive-behavioral therapy. Pharmacological approaches to the treatment of the primary insomnias have generally been recommended as short-term or adjunctive approaches to care. This has been in part due to longstanding concerns among physicians about the toxicity, tolerance, and addictive potential of many medications used for treating insomnia.

3 Medications for Treating Insomnia: Historical Use

A majority of the medications that have been used to treat insomnia exert primary effects at the GABA receptor and include barbiturates, barbiturate-like agents, and the benzodiazepines. Alcohol, probably the most widely used hypnotic medication, also exerts effects at the GABA receptor. The effects of these agents appear to be general and indiscriminate at each of the GABA-A α subtypes resulting in sedative and amnestic as well as hypnotic drug effects [4]. Historically, sedative/ hypnotics have been some of the most commonly prescribed drugs and initially utilized as anesthetics. Chloral Hydrate was the original “Mickey Finn” slipped into the drinks of unsuspecting marks for the purposes of criminal activity. Unfortunately, the LD-50 (potentially fatal dose) for Chloral Hydrate is quite close to the therapeutic dose, and murders rather than robberies were often the result. In the last century, barbiturates and barbiturate-like medications including methaqualone,

glutethimide, ethchlorovynol, and methypylon were commonly utilized for their hypnotic effects. Unfortunately, these medications, which are CNS depressants, pose a significant risk for lethal overdose, particularly when used concomitantly with alcohol. Marilyn Monroe, Elvis Presley, and Jim Morrison, among others, were celebrities who died from overdoses of CNS depressants, including sleeping pills [1, 5].

4 Current Medications Utilized in the Treatment of Insomnia

In patients with chronic insomnia, 22% of patients report using alcohol as a hypnotic [6]. Unfortunately, chronic use to induce sleep can result in tolerance, dependence, and diminished sleep efficiency and quality. When used in excess with other sedative/hypnotic agents, an overdose can be fatal. Chronic alcohol use has been shown to alter the activity of the GABA-A receptor complex [7]. This drug-induced imbalance of GABA inhibition has been proposed as a common feature characterizing alcohol, barbiturate, and benzodiazepine dependency disorders [8].

In the 1970s, benzodiazepines became available for the treatment of insomnia. These drugs have far less overdose danger than barbiturate-like medications. The many drugs in this class are best viewed therapeutically based on their pharmacodynamics (Table 1). Rapid onset of action is characteristic of flurazepam and triazolam, indicating that both these agents have excellent sleep-inducing effects. Flurazepam, like diazepam and clorazepate, has the characteristic of having active breakdown products. This results in an extraordinarily long, active half-life, which can approach 11 days. This effect, which is even more prolonged in the elderly, includes chronic sedation and decreased reaction times. These consequences in turn have been associated with increased auto accidents and falls with hip fractures [9, 10]. Withdrawal from these long-acting agents can be difficult, with an initial syndrome of insomnia followed by persistent anxiety that may extend beyond the half-life of the agent.

Benzodiazepines are REM sleep suppressant medications, and withdrawal often results in episodes of increased REM sleep (REMS rebound). REM sleep is known to have a role in learning and memory consolidation. For short-acting agents such as triazolam, this rebound occurs during the same night in which the medication was taken, and has been associated with daytime memory impairment, particularly at higher dosages [11]. Temazepam and estazolam have half-lives compatible with an 8-h period of sleep. Temazepam, because of its slower onset of action, is less efficacious as a sleep-inducing agent than other drugs in this class, which are used as hypnotics [12]. All benzodiazepines can result in respiratory depression in patients with pulmonary disease and tend to lose their sleep-inducing efficacy with prolonged use [13]. Such full GABA A agonists may induce receptor desensitization, leading to tolerance and subsequent withdrawal symptoms [14]. Chronic hypnotic medication use has been associated with development of mood disorders (depression) and hypnotic-dependent disorders of sleep [13]. Most medications

Table 1 Sedative hypnotics

Class	Drug	Sleep stage effects	Significant side effects
Benzodiazepines			
		Decreased amplitude stage 3 and 4 Increased stage 2 [all]	(1) Loss of effect with chronic use (2) Dependence (3) Respiratory depression Antegrade amnesia
Short onset Short ½ life <4 h	Triazolam [Halcion]	Shortened sleep latency In night REMS rebound	
Short onset Medium ½ life 8.5 h	Estazolam [Prosom]	Shortened sleep latency Decreased REMS	Daytime sleepiness
Short onset Long ½ life 50–110 h	Flurazepam [Dalmane]	Shortened sleep latency Decreased REMS Withdrawal REMS rebound	Daytime sleepiness, chronic buildup [Car accidents, hip fractures]
Medium onset Medium ½ life 7–10 h	Temezepam [Restoril] Clonazepam [Klonopin]	Decreased REMS	Daytime sleepiness, poor sleep induction
α1 GABA-A receptor agents			
(a) Short onset Medium ½ life	(a) Zolpidem [Ambien,	Shortened sleep latency, increased total sleep time, benzo effects with dose above that normally prescribed	Idiosyncratic: Daytime sleepiness, rebound insomnia, antegrade amnesia, parasomnias
(b) Short onset Short ½ life	Ambien-CR] and Eszopaclone (Lunesta)		
	(b) Zaleplon [Sonata]		
Other agents			
(1) Chloral hydrate	Chloral hydrate	(1) Short sleep latency, decreased REMS, withdrawal REMS rebound	(1) Low lethal dose, loss of effect with chronic use
(2) Barbiturates and barbiturate-like agents	Phenobarb-etc. Methaqualone Glutethimide Ethechloro-vynol Methylprylon	(2) REMS suppression, short sleep latency, decreased REMS, withdrawal REMS rebound	(2) Addiction low lethal dose, loss of effect with chronic use
(3) Sedating antihistamines H 1-blockers	diphenhydramine	(3) Decreased sleep latency in some patients	(3) Daytime sedation, anti-cholinergic effects, falls and cognitive impairment in the elderly
Melatonin agonists	Ramelteon	Shortened sleep latency	Neurohormonal interactions

known to induce somnolence as an effect or sideeffect have similar effects on both sleep and waking, potentially leading to deficits in waking function (Table 2).

The newer hypnotics, zolpidem, zaleplon, and eszopiclone, are benzodiazepine-like agents exerting primary effects at the α1 subtype GABA-A receptor. These

Table 2 Medication types reported in clinical trials and case reports to have sleepiness as an effect or side effect

Medication class	Neurochemical basis for sleepiness
Antihistamines	Histaminine receptor blockade
Antiparkinsonian agents	Dopamine receptor agonists
Antimuscarinic/antispasmodic	Varied effects
Skeletal muscle relaxants	Varied effects
Alpha-adrenergic blocking agents	Alpha-1 adrenergic antagonists
Beta-adrenergic blocking agents	Beta adrenergic antagonists
Opiate agonists	Opioid receptor agonists (general CNS depression)
Opiate partial agonists	Opioid receptor agonists (general CNS depression)
Anticonvulsants	
Barbiturates	GABA receptor agonists
Benzodiazepines	GABA receptor agonists
Hydantoins	General effects?
Succinimides	General effects?
Other	Varied effects including GABA potentiation
Antidepressants	
MAOI	norepinephrine, 5HT, and dopamine effects
Tricyclic	Acetylcholine blockade, norepinephrine and 5HT uptake inhibition
SSRI	5HT uptake inhibition
Others	5HT, dopamine, and norepinephrine effects
Antipsychotics	Dopamine receptor blockage, varied effects on histaminic, cholinergic and alpha adrenergic receptors
Barbiturates	GABA agonists
Benzodiazepines	GABA agonists
Anxiolytics, misc. sedative and hypnotics	GABA agonists, varied effects
Antitussives	General?
Antidiarrhea agents	Opioid, general?
Antiemetics	Antihistamine and varied effects
Genitourinary smooth muscle relaxants	General?

agents have excellent hypnotic efficacy with fewer side effects and residual next day effects than traditional benzodiazepines [15]. Although any agent used to induce sleep can result in a dependence on that agent to induce sleep, abuse potential for these agents is minimal [16]. Idiosyncratic reactions of persistent daytime somnolence and/or memory loss have been reported in some patients. Tachyphylaxis is unusual with the use of these agents and sleep is minimally altered. Zolpidem, the most widely used of the newer medications, has a 1.5–4.0 h half-life, but it is available in an extended release form. Abrupt discontinuation can be associated with withdrawal symptoms and rebound insomnia [17]. Eszopiclone has a 6–8 h half-life, has no reported REMS rebound on discontinuation, and has been used long term without adverse consequence in clinical trials [18]. Zaleplon is shorter acting (3–4 h) and can be dosed during the night of sleep [19]. The recent development of these medications, which have high efficacy, minimal toxicity, lack

of tolerance, and minimal addictive potential, has led to recommendations that they be used long term for the treatment of chronic insomnia [20, 21].

In the last 30 years, although the drugs for treatment of insomnia have become safer, the number of sedative/hypnotics prescribed in the United States has declined. This decrease most likely reflects both the public's and medical community's concerns regarding the side effects, limitations, and cost of the available hypnotic drugs. Most hypnotic medications, in general, can be safely utilized on a short-term basis for the treatment of transient insomnia. Chronic hypnotic use with the newer nonbenzodiazepine hypnotics can be justified and is indicated if medication use leads to improvement in waking performance [11]. These newer hypnotic agents are less likely to have deleterious side effects than most OTC treatments for insomnia [22].

5 Nonprescription Sedating Agents

Over the counter sleeping pills contain sedating antihistamines, usually diphenhydramine. These agents are varyingly effective but may result in daytime sleepiness, cognitive impairment, and anticholinergic effects, which persist into the day following their use, thus affecting driving performance [23]. These agents are not recommended for use in the elderly [24]. Seizure thresholds can be lowered in epileptic patients who use these agents. Both alcohol and sedating antihistamines are associated with decreased performance on daytime driving tests and an increase in automobile accidents. Sedation is infrequent with H₂ antagonists (e.g., cimetidine, ranitidine, famotidine, and nizatidine), but somnolence as a side effect is evidently reproducible in susceptible individuals [25]. The side effect profiles of the newer sedative-hypnotics are generally more benign than those of the sedating antihistamines [26].

In addition to the sedating antihistamines, a variety of nonprescription and herbal agents are marketed as hypnotics. Melatonin is a neural hormone effective in resetting circadian rhythms of sleep and body core temperature through its actions on the suprachiasmatic nucleus. For individuals with insomnia secondary to disruptions in circadian rhythms, melatonin can act as a hypnotic and is a useful adjunct to treatment that often includes cognitive therapies, light exposure, and other hypnotics. Tryptophan has a history of known efficacy in the treatment of chronic insomnia. In the late 1980s, use of this agent was associated with severe eosinophilia that was lethally toxic in some cases. This agent was removed from the market, despite speculation that the toxicity was not secondary to the drug itself but to deficiencies in the preparation process. Tryptophan is again available in some dietary supplements, yet is most safely utilized in a bedtime snack of foods with known tryptophan content such as milk, bananas, and turkey. Kava, considered a drug of abuse in some cultures, has been used for insomnia but has shown potential for hepatic toxicity in some patients. Kava is postulated to facilitate GABAergic transmission [27]. The best data supporting the sedative effect of a herbal agent is

for Valerian [28]. Evidence supporting the hypnotic efficacy of other herbal agents including chamomile, passionflower, and skullcap is limited.

6 Antidepressants

Sedating antidepressants are often used to treat insomnia. A significant percentage of individuals with chronic insomnia and/or daytime sleepiness also have depressive symptoms. Chronic insomnia itself can predispose patients to develop depression [29]. Depression associated with insomnia is likely a different diagnostic entity than depression without insomnia, and treatment of the former with nonsedating antidepressants may produce no improvement in sleep even when the underlying depression resolves [30]. Use of antidepressants is limited by side effects (anticholinergic effects, daytime hangover, etc.) and danger with overdose (particularly the tricyclics) [1, 31, 32]. Sedating antidepressants include the tricyclics (amitryptiline, imipramine, nortryptiline, etc.); trazadone, mirtazapine, and nefazodone. The selective serotonin reuptake inhibitors (SSRI's) have a tendency to induce insomnia; however, in some patients, paroxetine may induce mild sedation [25]. Depression-related insomnia responds to sedating antidepressants more rapidly and with lower doses compared to other symptoms of depression [32]. In patients with insomnia and concomitant depression, antidepressants are often used in combination with sedative-hypnotic medications [33]. Use of sedating antidepressants has been associated with declines in daytime performance, driving test performance, and an increased potential for involvement in motor vehicular accidents [34].

7 Psychoactive Medication Effects on Sleep Stages and EEG

Different classes of psychoactive medications induce pharmacologically characteristic electrophysiologic changes in sleep [35, 36]. Medication-induced changes in electrophysiology can lead to an increase in symptoms occurring during those specific sleep/dream states. For example, insomnia and nightmares are associated with the REM sleep rebound that occurs after discontinuation of REM suppressant drugs (i.e., alcohol, barbiturates, benzodiazepines). Medications such as lithium and GABA-hydroxy-butyrate (sodium oxybate) that can cause an increase in deep sleep can induce the occurrence of arousal disorders such as somnambulism [36, 37]. The influence of psychoactive medications on sleep states has a positive side as well. For example, REM sleep suppressive medications can be useful adjuncts in the treatment of REM sleep parasomnias and sleep stage-specific symptoms. Both benzodiazepines and antidepressants can be used to decrease REM sleep. Similarly, arousal disorders can be treated with medications affecting deep sleep

(benzodiazepines and others) [36]. The benzodiazepine clonazepam is the medication most commonly utilized in the treatment of parasomnias, particularly in REM behavior disorder [38].

8 Nonpharmacologic Therapies for Insomnia

Nonpharmacologic approaches have been shown to be effective in treatment for primary, secondary, and comorbid insomnia, sometimes in lieu of a pharmacologic approach but frequently as a part of a treatment plan. The overall approach is called “Cognitive Behavioral Therapy” (CBT), which involves several modalities including as basic an approach as good sleep hygiene as well as stimulus control, sleep restriction, and relaxation techniques. Sleep hygiene addresses both circadian and homeostatic sleep patterns. Establishing a regular sleep pattern, particularly a fixed wake up time, and avoiding naps is the first step. It is important to create a proper sleep environment free of external stimuli, quiet, cool, and dark. Daytime activities including morning exposure to bright light and exercise at least several hours before bedtime are helpful. An overall healthy lifestyle with special attention to diet and avoidance of stimulants is part of this process.

Stimulus control therapy involves a reeducation of the patient with instructions that further reinforce good sleep hygiene and also ask the patient to avoid going to bed until he/she is sleepy and to actually get out of bed if he/she is unable to fall asleep. It also advises them that the bedroom and bed are to be used only for sleep and intimacy and not to become a room for reading, watching TV, sewing, etc.

Sleep restriction therapy is a more aggressive approach that for many insomniacs is counter intuitive. It involves delaying bedtime and fixing wake time so that the initial sleep opportunity is more in line with the insomniacs’ perception of their current actual sleep time. Thus, if they feel they are only sleeping 5 h and need a wake time of 7 a.m., their bedtime becomes 2 a.m. As they achieve greater sleep efficiency for this period, they can gradually advance the sleep time, with an eventual goal of near the desired adult sleep opportunity of 7–8 h.

Relaxation training is aimed at reducing the heightened arousal that insomnia patients often experience. Various techniques involving visualization in combination with progressive muscle relaxation are commonly used, but other approaches work equally well. The goal is to reduce intrusive ideas. As a part of this program, the patient is asked to actually keep some sort of diary before going to bed and to “download” everything on their mind that might intrude when they attempt to fall asleep. This approach can be helpful in preventing rumination about the next day’s events, plans, etc.

Cognitive therapy is primarily an educational approach aimed at reducing misconceptions about sleep and the anxiety produced about sleep by these misconceptions. Among the “rules” are teaching the patient to never “try” to sleep but rather to allow themselves to sleep. Educating them regarding the fact that a single night of poor sleep does not have to predict subsequent nights of insomnia is important

[39–41]. These approaches have been well reviewed and suggest that secondary or comorbid insomnia as well as primary insomnia respond to CBT. Of note is the fact that while CBT is usually slower to work and often requires several sessions of working with the provider, its effects have proven to be longer lasting and freer of relapse than medication approaches to the treatment of insomnia [40, 42, 43]. In fact, the effect of this approach has been shown to be augmented with time with respect to sleep latency and total sleep time. The benefits can be seen with single modalities of cognitive and/or behavioral treatment but appear more robust with combination therapies, including the augmentation of such an approach with the use of hypnotic medications [41].

9 Secondary Insomnia

The term secondary insomnia has historically been applied to patients with insomnia who also have a diagnosis of either a medical or psychiatric condition or a primary sleep disorder. Until 2005, NIH guidelines regarded such insomnia as being a consequence of the primary diagnosis. This led to recommendations that indicated the key to resolving the problem was to treat the primary or underlying condition with the assumption that this would in turn lead to resolution of the insomnia. In 2005, the NIH convened another “State of the Science” conference to review the manifestations and management of chronic insomnia [44]. The committee concluded that most cases of insomnia are comorbid with other conditions. The concern over continuing to use the term “secondary” insomnia is that in many cases, we do not have clear proof of cause and effect, and of greater concern was the possibility that use of the term might lead to undertreatment of the insomnia. The recommendation to view insomnia as comorbid has led to a shift in treatment paradigms. While identification and treatment of the “primary” condition remains a priority, concurrent treatment of insomnia is now viewed as desirable. In general, treatment of comorbid insomnia is now essentially the same as treating primary insomnia with a growing number of studies confirming that this approach is effective [42]. Treatment of insomnia will in many cases ameliorate the symptoms of the “primary” or comorbid condition.

10 Psychiatric Disorders and Insomnia

Psychiatric disorders have been strongly linked to the presence of insomnia. Diagnoses commonly comorbid with insomnia include major depression, bipolar mood disorder, anxiety disorders, psychotic disorders, and amnestic disorders such as Alzheimer’s disease. Estimates of the incidence of insomnia with these diagnoses are in the 50–75% range [42, 45]. The most common association is with depression. Insomnia and depression seem to have a circular or bidirectional relationship. There are several studies that show that insomnia patients are at risk

for developing depression. Insomnia may be the first symptom of patients who develop depression. In a large study of young adults over a period of 20 years, 2 weeks of insomnia or longer predicted major depressive episodes and major depressive disorders. 17–50% developed a major depressive episode. Recurrent insomnia may be the earliest sign that a patient in remission from their depression is at risk of a relapse [29, 46–48]. There is also an increased risk for developing anxiety or a substance abuse disorder [49]. In addition to the evidence identifying persistent insomnia as a risk factor for new psychiatric disorders, findings have suggested that chronic insomnia problems may also contribute to the persistence of depressive symptoms. This issue is of particular importance in light of the significant rate of residual sleep disturbance in persons who have been otherwise successfully treated for depression. Insomnia may persist after treatment of depression and is considered the most refractory symptom of depression. Drawing on data from a large interventional study of enhanced care for depressed elderly persons, the investigators found that persistent insomnia was associated with a 1.8–3.5 times greater likelihood of remaining depressed, compared with the population without continued sleep disturbance [50]. The relationship between insomnia and depression is further complicated by the fact that many common antidepressants, especially the selective serotonin reuptake inhibitors (SSRI's), may be associated with disturbed sleep. Fluoxetine, imipramine, and protriptylene have all been identified as at risk for this side effect [51].

11 Treatment of Secondary (Comorbid) Insomnia Associated with Psychiatric Disorders

Traditionally, the treatment of insomnia associated with another diagnosis has been to treat the “underlying” or primary condition with the assumption that this would lead to resolution of the insomnia. This is clearly not the case in many instances. In patients with insomnia and a psychiatric diagnosis, treatment options include those also used for primary insomnia. This could include either pharmacologic treatment, cognitive behavior treatment (psychological and behavioral), or a combination of both. Medications have also been used on a “prn” basis with benefit perceived by the patients. There have been relatively few studies that look at improvement in daytime function, though improved sleep would be expected to result. Where it has been examined, the data support the beneficial effect [52].

Eszopiclone has been studied in patients with major depression along with simultaneous use of fluoxetine [53]. The combination was well-tolerated and resulted in a rapid improvement in sleep. Of note, there was also a more rapid and larger antidepressant response. This does not suggest an antidepressant effect of eszopiclone but rather that improved sleep has a beneficial effect on depression. This makes a strong case for a combined approach to treatment, i.e., simultaneous treatment of the two entities rather than the traditional approach of waiting for insomnia to improve as a result of treating depression. Combining antidepressant

treatment with cognitive-behavioral therapy for insomnia has also been shown to be superior to the antidepressants alone, both in terms of depression outcome (61.5 vs. 33.3% remission, respectively) and insomnia outcome (50 vs. 7.7% remission, respectively) [43]. A similar result occurred with the use of eszopiclone with escitalopram for generalized anxiety disorder compared to the escitalopram alone [54]. These studies suggest that there may be a major shift in the approach to treating depression since insomnia is so prevalent in depressed patients. Concurrent treatment of both the insomnia and the psychiatric condition at the outset of treatment may become the norm.

11.1 Melatonin Receptor Agonists

The only non-BZRA approved as a hypnotic is the melatonin receptor agonist ramelteon. Although its use has been studied in primary insomnia, there is currently no data about its role in secondary insomnia. Melatonin has been used as a hypnotic with inconsistent results, although in a study comparing the effects of variable melatonin dosing vs. placebo for sleep-onset problems in chronic schizophrenic patients, reduced awakenings and increased total sleep time were demonstrated in the melatonin group; however, results for the primary target, sleep-onset latency, were mixed. Daytime mood and functioning also showed improvement.

12 Primary Sleep Disorders Associated with Insomnia

12.1 Sleep Apnea

Sleep apnea can occur with symptoms of insomnia. There is little in the literature to suggest that the sleep apnea or the insomnia should be dealt with differently when they are present as the primary diagnosis and not comorbid [55]. The current approach should deal with both using standard treatment. In a recent review of this subject, one article dealing with the use of CBT for the treatment of insomnia in sleep apnea patients showed a high degree of success as it does in insomnia comorbid with psychiatric diagnosis. This suggests that identification of sleep apnea should be considered in all patients with insomnia and that treatment of OSA will improve insomnia in at least some of these patients.

12.2 Circadian Rhythm Sleep Disorders

Sleep regulation is complex, and a full consideration of this topic is beyond the scope of this chapter. In general, there is an interaction between the homeostatic and the endogenous circadian processes. The homeostatic process is sleep need

or drive that accumulates through the wakeful hours. This therefore is impacted by a patient's sleep hygiene, life style, work times, and overall patient behavioral differences. On the other hand, circadian processes of sleep are largely controlled by the suprachiasmatic nucleus (SCN) in the hypothalamus, with the SCN promoting wakefulness. The internal human clock responds to external factors with the greatest influence being exposure to light and darkness. The other element, which is important to the timing of the sleep wake cycle, is the endogenously produced melatonin, which is produced in the pineal gland in response to signals from the SCN. In addition to its effects on the sleep wake cycle, the SCN regulates body temperature and numerous other processes that have circadian rhythms [56]. Sleep disorders can also result from a misalignment of the approximately 24 h endogenous circadian rhythm and the "normal" 24 h day night cycle.

12.3 Delayed Sleep Phase Syndrome

One of the commonest of these disorders is the Delayed Sleep Phase Syndrome (DSPS). Patients with this syndrome have difficulty falling asleep at the desired bedtime, often falling asleep between 2 and 6 a.m. and then, if their life style permits, sleeping approximately a normal 8 h, awakening at between 10 a.m. and 2 p.m. With the demands of school or work and the need to rise earlier, these patients present a clinical picture that is similar to that of severe chronic sleep onset insomnia. They suffer from chronic insufficient sleep time with all its daytime consequences. It is most prevalent in adolescents and young adults but can be seen in older patients. The pathophysiology of this syndrome has not yet been characterized, but hypothesized causal factors include a long endogenous circadian period, hypersensitivity to evening light, and genetic mutations [57]. Bright light therapy has been one of the most commonly used therapeutic approaches to treating DSPS, and while widely reported to be effective, it has been the subject of only a limited amount of systematic research. Bright light therapy involves exposure to bright light at the proper time in the circadian phase response curve. In the case of delayed phase syndrome, this is after the nadir of body temperature. Treatment with 10,000 lux for 30 min has become a common recommendation. In patients with severe phase delay, identifying the temperature nadir may be difficult, and light administered prior to the nadir can actually further delay the circadian rhythm [58, 59].

Timed melatonin administration has also been used in many of these patients, although studies vary with recommendations of dosage and timing. Administration of melatonin in doses ranging from 0.3 to 5 mg have been reported. Reports of the optimal time to administer melatonin also vary, but treatment in the early evening 5–7 h before sleep time, before the Dim Light Melatonin Onset (DLMO), or 12+ h before the temper nadir have been found effective. There seems to be consensus of the efficacy with remaining issues as to ideal dose and timing. Melatonin can be

soporific, and thus caution using this approach has been recommended, especially if the patient has activities to perform that require full alertness [57–59].

12.4 Advanced Sleep Phase Syndrome

Advanced sleep phase syndrome is the mirror image of DSPS, with patients experiencing both sleep onset and awakenings that are several hours earlier than desired, but with the total sleep period remaining fairly normal. This is much less common than DSPS and tends to occur more in middle aged to elderly adults. Treatment options are similar to those for delayed phase syndrome, with timing of treatment designed to delay rather than advance the circadian rhythm.

12.5 Shift Work Sleep Disorder

Shift work relates to work that is carried out at nonstandard times of the day and night. The degree of shift in sleep and work time varies tremendously, but approximately 20% of the work force in the United States has some degree of shift work. The disorder refers to the symptoms that occur because of the need to work during the usual sleep period. The most significant problems occur with “graveyard” and early morning shifts. The symptoms include insomnia, sleepiness, and poor performance. Patients frequently have difficulty with sleep onset and sleep maintenance as well as sleepiness on the job. Shift workers on the whole get as much as 10 h a week less sleep than do their regular shift colleagues [20, 24]. Treatment options are complex. No technique has completely resolved the problems of impaired performance or personal health effects. There is evidence that planned napping before a shift or during the shift results in improved alertness on the job [24]. Timed light exposure can benefit the worker. This may include bright light exposure early in the night shift and ending some time before the end of the shift. While it is difficult to study this in a controlled way, there are several studies that seem to indicate that this intervention has beneficial effects.

Medications have also been tried with some success. Melatonin has been used to help more rapidly the shift workers’ circadian rhythms as required. When taken before bedtime in the early morning, it can improve sleep quality. Hypnotics clearly help improve sleep during off hours when sleep time occurs during the circadian period when the individual is normally awake and alert. The most convincing data supports the use of stimulants to help the workers’ alertness during night shift work. Modafanil has been studied and is successful enough to have gained FDA approval for this indication. Caffeine has also been used with success, though there are few controlled studies. It has also been suggested that CBT be offered to shift workers. While recognizing the challenge of the obvious inability to keep the same bedtime and wake-time, additional recommendations include advocacy of practicing good sleep hygiene [20, 22, 24].

12.6 Jet Lag Disorder

Jet lag is the temporary mismatch of circadian and homeostatic sleep patterns with social and environmental cues resulting from rapid travel across multiple time zones. It is in many ways similar, on a short-term basis, to shift work. Symptoms include sleep onset insomnia, daytime sleepiness, and reduced performance. There is conflicting data on whether younger or older travelers suffer more, but there is a suggestion that older individuals may actually deal more adaptively with jet lag [72].

Approaches similar to those for delayed and advanced sleep phase syndrome can also be useful in speeding up the adjustment to a new circadian rhythm. Properly timed bright light exposure both before leaving and after the journey can help but can be difficult to time. Studies on actual travelers are few, but those that have been carried out, including those based on laboratory models of jet lag, suggest that these measures are beneficial. Similarly, there is data indicating that melatonin, in immediate release doses, which have ranged from 0.5 to 5 mg, can help to speed up the adjustment to the new time zone. In the AASM review, caution is also recommended if alcohol is being ingested at the same time. As with shift work, the use of stimulants would seem logical, but there are few data to support this suggestion. Caffeine has been the focus of some limited study and may be beneficial [57, 59, 60].

12.7 Restless Legs Syndrome/Periodic Leg Movement Disorder

Restless legs syndrome (RLS) is a common neurologic condition marked by the urge to move the legs, although other parts of the body are also affected, occurring primarily at rest in the evening or bedtime. The minimum criteria for making the diagnosis include (1) An urge to move the legs, usually accompanied or caused by uncomfortable and unpleasant sensations in the legs; (2) The urge to move or unpleasant sensations, which begin or worsen with rest or inactivity; (3) The urge to move or unpleasant sensations, which are partially or totally relieved by movement; (4) The urge to move or unpleasant sensations, which are worse or only occur in the evening or night [61]. Some form of sleep disruption is present in as many as 76% of the patients with the syndrome [62]. RLS is associated with complaints of decreased quality of life, which seems to be out of proportion to the severity of the individual complaints. Daytime consequences of the sleep disruption has been reported. There are, however, a surprising number of patients who do not have as much daytime sleepiness as would be expected. This is despite the fact that there can be a fairly marked sleep loss [63].

The treatment of the sleep disruption, primarily sleep onset, in RLS relies predominately on the treatment of the RLS rather than the sleep disorder. Dopaminergic agonists have become the primary initial treatment for RLS. Pramipexole and ropinirole have both received FDA approval for this indication and are used in

doses that are lower relative to their use for Parkinson's Disease. Pramipexole is used in a range of 0.125–2 mg and ropinirole at 0.25–4 mg. Benzodiazepines have also been used. Historically, these have included clonazepam as well as temazepam. There are no recent studies of the efficacy of these, but historically, they have been useful and still have a role when side effects limit the use of the dopaminergic agents, or in combination with them in refractory cases. When lack of response or side effects are still present, lower potency opioids such as codeine or oxycodone, as well as the antiepileptic drugs carbamazepine and gabapentin, have been found effective [64]. Recently, a new nondopaminergic compound, XP13512, at a dose of 1,200 mg, was studied and found effective. This is a modified form of gabapentin, which is converted to gabapentin after ingestion but avoids the variable absorption that can occur with the parent compound [65]. Some types of medication including antidepressants, antiemetics, and antihistamines can exacerbate RLS in some patients [64].

13 Sleep and Medical Disorders

Almost any medical condition can, in certain circumstances, interfere with and be comorbid with sleep. As in the previous conditions, there is growing evidence for the bidirectional nature of this comorbidity. Treatment of sleep disruption in the presence of other medical conditions is therefore not different than has been described above. The key is recognition of the sleep problem as an independent issue, which then can trigger an appropriate response. A few specific medical conditions with a strong relationship to insomnia are appropriate to consider.

13.1 *Pain and Sleep*

Chronic pain leads to poor sleep in a majority of patients suffering from it. As the incidence of chronic pain is estimated at, at least, 11%, this represents a large population. Pain can be an acute or chronic part of a broad range of medical ailments, most commonly, cancer, rheumatologic disorders, and headache. Acute pain can disrupt sleep, but once the pain resolves, the sleep issue usually also resolves. Chronic pain and sleep disruption seem to lead to a vicious cycle of pain causing poor sleep, and poor sleep leading to greater pain. Management of this is suggested in an algorithm that recommends diagnosis of the sleep problem, emphasis on sleep hygiene, and then CBT techniques followed by pharmacologic interventions. These include medications for both pain and insomnia. There is evidence that CBT can improve insomnia in these patients [40, 66]. In rheumatologic disorders, treatment of sleep with hypnotics or sedating antidepressants improves sleep as well as pain tolerance. Fibromyalgia has been associated with decreased slow wave sleep, characterized by frequent alpha intrusion, and reduced sleep quality and quantity.

13.2 Sleep in Other Medical Conditions

Patients with respiratory problems can also suffer from disrupted sleep. COPD patients frequently have fragmented sleep. This can improve with oxygen if hypoxia is part of the problem. About one-third of asthma patients who are poorly controlled have nocturnal asthma attacks that interfere with their sleep and may lead to daytime symptoms. Patients with gastroesophageal reflux often have sleep disruption associated with the reflux. In addition, reflux can trigger asthma attacks in vulnerable patients. Patients with end stage renal disease suffer from a variety of sleep disorders with a very high prevalence. These can include insomnia, sleep apnea, and a high incidence of secondary RLS. Menopause is associated with insomnia, which does respond to treatment with hormones as well as treatment using a hypnotic [67, 68].

14 Insomnia and the GABA Receptor

All of us spend at least a third of our lives in sleep. Chronic difficulty in sleeping affects at least 14% of the population and leads to impairments in waking performance and quality of life [1, 2]. As noted above, insomnia can be a primary disorder, but also occurs secondary to a wide spectrum of medical, psychiatric, and sleep disorders. It is remarkable that most drugs affecting this global CNS cognitive state affect one primary neurotransmitter receptor system. Almost all medications classified as hypnotics exert behavioral effects at the GABA receptor. And if that were not enough, the psychopharmacologic effects exerted at this receptor extend beyond sleep. The behavioral consequences of pharmacologic modulation of the GABA (Benzodiazepine) receptor include anxiolysis, sedation, amnesia, myorelaxation, anesthesia, pain relief, tolerance, possible abuse potential, and interactions with alcohol [69–71]. These various effects are modulated at specific GABA receptor subtypes with gating properties varying markedly depending on subunit combinations [14]. Agents with general and indiscriminate effects on the GABA receptor induce a combination of effects, often with negative and detrimental consequences. The newest, safest, and most efficacious of the hypnotics exert primary effects at the $\alpha 1$ subtype GABA A receptor [4]. While this paradigm is well described as a site for behavioral hypnotic medication effects, the subunit stoichiometry and specific regional distributions of various receptor sites in the CNS are still being elucidated [14, 15]. Sleep is a complex, global state. Our understanding of its neurochemistry, like our understanding of its electrophysiology, function, effects on memory and mind, and appropriate treatment for its disorders, remains incomplete.

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Zolpidem in the Treatment of Adult and Elderly Primary Insomnia Patients

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Abstract The present section discusses the place of zolpidem in the treatment of primary insomnia including its new delivery formulations. The drug mechanism of action is first briefly reviewed. Then the pharmacokinetic, metabolic, and therapeutic properties of zolpidem are examined with a special focus on new delivery formulation such as extended-release and sublingual forms. The sleep-inducing effect of zolpidem has been convincingly demonstrated in various population and settings. Efficacy results coming from well-designed placebo-controlled clinical trials in chronic and/or primary insomnia show that the drug improves both subjective and objective sleep parameters such as sleep latency, wake after sleep onset, number of awakenings, total sleep time, sleep efficiency, sleep quality, and daytime functioning. Studies investigating intermittent and “as needed” administration of zolpidem as well as their combination with cognitive behavioral therapy indicate the usefulness of these approaches. Finally, zolpidem tolerability data are discussed and it is concluded that, when administered according to the manufacturer’s prescribing information, zolpidem is well tolerated and associated with minimal rebound insomnia and a low propensity to cause next-day residual effects, drug abuse, or dependence.

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1 Introduction

Insomnia is the subjective complaint of poor sleep, either in terms of duration or quality, and sometimes that sleep is not refreshing. Although individuals vary widely in their sleep needs and practices, it is generally admitted that insomnia is characterized by a sleep onset latency of more than 30 min, a total sleep time of less than 6 h, numerous nocturnal awakenings, early morning awakenings with inability to resume sleep, or nonrestorative sleep. According to these characteristics, most adults have experienced insomnia or sleeplessness at one time or another in their lives. For instance, epidemiological studies indicate that insomnia is one of the most pervasive health disturbances in the general population with an estimated prevalence varying from 10 to nearly 60% depending in part on the use of varying definitions and data-collection methodologies [1]. In the National Sleep Foundation's "Sleep in America Poll 2005" survey, approximately 54% of Americans indicate that they experienced at least one of the four symptoms of insomnia (difficulty falling asleep, awake at night, waking too early, waking up unrefreshed) at least a few nights each week and 33% experienced at least one of the four symptoms every night or almost every night during the previous year.

Insomnia is considered as a diagnosis when difficulty with the initiation, duration, maintenance, or quality of sleep occurs repeatedly (i.e., at least 1 month) despite adequate time and opportunity for sleep and results in some form of daytime impairment. The estimated prevalence of insomnia falls below 10% when such diagnostic criteria are applied; for instance, the prevalence of insomnia diagnoses according to the DSM-IV classification has been estimated at 6% [1]. Individuals reporting disturbed sleep are more likely to report emotional distress and recurrent health problems [2–4]. Indeed, about 17% of patients with insomnia feel that their insomnia has serious impact on their lives [5], and it was shown, in a prospective study, that poor sleepers were less effective in their work, less likely to receive promotion, and more likely to be demoted, discharged, or not reenlisted and that these problems are the consequence and not the cause of their sleep difficulties [6]. This is not surprising since it has been shown that sleep deprivation has many impacts on the daytime life of healthy subjects; alertness, attention, concentration, cognitive abilities, memory, mood, and pain threshold have all been found impaired, even with a sleep deprivation of as little as 1–2 h per night [7–9].

Insomnia may be caused by a host of different reasons. Transient (less than 1 week) and short-term (less than 3 weeks) insomnia are often caused by situational factors such as inappropriate sleep conditions (excessive noise, inadequate temperature), stressful situation (exam preparation, work pressure, familial difficulty, loss of a loved one, hospitalization), or circadian misalignment (jet lag, changes in shift work). Chronic insomnia is often comorbid with a chronic medical condition or a psychiatric illness such as mood or anxiety disorder, but there are also chronic primary forms of insomnia such as psychophysiological insomnia or idiopathic insomnia. The treatment of chronic comorbid insomnia is often challenging

because the comorbid condition can in turn be aggravated by a loss of restorative sleep [10, 11].

Consequently, in cases where insomnia symptoms are linked to other physical or mental complaints, the treatment will aim to address a complex combination of symptoms, whereas in cases of primary insomnia, the treatment will aim to reduce insomnia symptoms alone.

It has been estimated that less than 15% of those with severe insomnia receive any treatment [12]. Most patients initiate treatment with various nonprescription therapies of unknown risk and benefit (such as OTC sedating antihistamines, herbal or dietary supplements) or rely on alcohol to attempt to address their condition. Both pharmacological and nonpharmacological approaches are valuable treatment options for primary insomnia. However, the use of nonpharmacological treatments that include a variety of behavioral sleep management techniques (sleep hygiene measures, cognitive therapies, relaxation, sleep restriction, etc.) is limited by lack of trained providers, cost, third party reimbursement, and understanding of the treatment methods [13].

Ideally, effective pharmacological treatment for insomnia should normalize sleep patterns but, importantly, not impair next day function. First-generation hypnotics such as barbiturates, carbamates, chloral hydrate, and methaqualone were effective in inducing sleep but put patients at risk not only for next-day residual effects but also for developing tolerance, experiencing withdrawal effects, or for suffering from fatal overdose. The modern era of pharmacology for insomnia began in the seventies when benzodiazepines were introduced and rapidly increased in popularity because of their efficacy and better safety compared to first-generation hypnotics. Recent years have seen a decline of the prescriptions of benzodiazepines that coincided with the introduction of nonbenzodiazepine hypnotics, such as cyclopyrrolones (zopiclone and eszopiclone), imidazopyridines (zolpidem), pyrazolopyrimidine (zaleplon and indiplon), melatonin receptor agonists (ramelteon and tamiselteon), or certain antidepressant drugs.

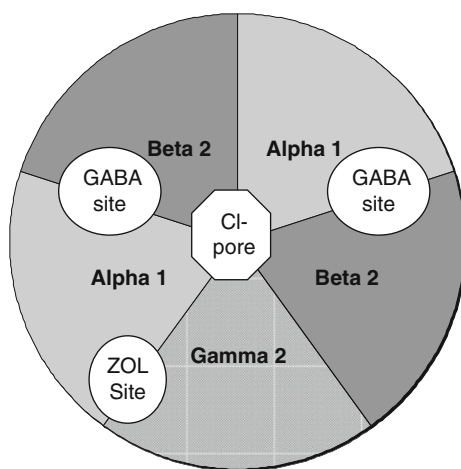
Among those, zolpidem rapidly became the most widely used hypnotic drug. Success relates to the fact that zolpidem has proved to be a suitable hypnotic, especially with regard to efficacy in sleep initiation, with a low incidence of side effects. As will be discussed below, advantages of zolpidem over benzodiazepines are its short half-life in the circulation and its more selective GABA_A receptor binding profile. Historically, the therapeutic potential of the imidazopyridine compound zolpidem for the treatment of insomnia was recognized in the eighties, soon after it was synthesized by Synthélabo Recherche [14–15]. The pharmacokinetic profile of the initial formulation of zolpidem (immediate-release-IR zolpidem) was one of the short-acting drugs with a peak plasma concentration attained at about 50 min after the usual dosage of 10 mg and a maximum of activity around 1.5 h followed by a rapid decline [16]. Recently, alternate delivery forms have been developed such as extended-release zolpidem (Ambien CRTM, Sanofi-Aventis), sublingual zolpidem (EdluarTM, Meda and IntermezzoTM, Transcept), and zolpidem oral spray (ZolpimistTM, Novadel Pharma). The present chapter of this volume

is devoted to discuss the place of zolpidem, including its new delivery formulations, in the treatment of primary insomnia in the light of recent studies.

2 GABA_A Receptor Subtypes and the Mechanism of Action of Zolpidem

The GABA_A receptor is an ionotropic receptor and ligand-gated ion channel that mediates the majority of the inhibitory neurotransmission pathways in the brain. The receptor consists of five subunits, the most common combination being two α s, two β s, and one γ subunit [17] that are arranged around a central pore that selectively conducts chloride anion (Fig. 1). Numerous subunit isoforms are evidenced (for instance, in humans, six subtypes are described for the α subunit, three for the β subunit, and three for the γ subunit), whose combination determines the GABA_A receptor's properties, including its agonist affinity [18]. The receptor binds two GABA molecules, at the interface between an α and a β subunit. Once bound to GABA, the protein receptor changes conformation within the membrane and opens the chloride pore allowing chloride anions to enter the neuron and to produce an hyperpolarization that decreases neuronal excitability [18]. The active sites of the GABA_A receptor also bind several drugs such as muscimol, gaboxadol, and bicuculline. The receptor protein complex also contains distinct allosteric binding sites, which modulate the activity of the receptor indirectly and are the targets of various other drugs, including the benzodiazepines, barbiturates, and ethanol among others [19]. For instance, benzodiazepines increase the affinity of the receptor to GABA by binding at a site located at the interface between the α - and γ -subunits of α - and γ -subunit containing GABA_A receptors [20].

Fig. 1 Schematic diagram of a GABA_A receptor protein complex, which illustrates the five combined subunits that form the protein $[(\alpha_1)_2(\beta_2)_2(\gamma_2)]$, the chloride (Cl^-) ion channel pore, the two GABA active binding sites at the α_1 and β_2 interfaces, and the zolpidem (ZOL) allosteric binding site at the α_1 and γ_2 interface



Zolpidem differs from benzodiazepine derivatives because of its selectively higher affinity for a subclass of benzodiazepine binding site to which nomenclature first referred as ω_1 [21]. Further identification of the heteropentameric structure of the GABA_A receptor complex, by mutation analyses and antibodies inactivation, evidenced that α_1 subunit was corresponding to the ω_1 site [22, 23]. Indeed, the affinity of zolpidem to α_1 -containing GABA_A (α_1 -GABA_A) receptors is about 10-fold higher than to those containing α_2 or α_3 subunits, and zolpidem has no appreciable affinity for α_5 subunit-containing receptors [24–29]. Intrinsic efficacy on GABA shift or chlorine influx is high for zolpidem, which can therefore be described as a full agonist. The molecular location of zolpidem binding site seems to be the interface between the α_1 and the γ_2 interfaces (see Fig. 1), and the affinity is lost when some residues of these subunits are missing [30–32]. The subunit affinity carries some specificity for pharmacological actions, and α_1 is responsible for the sedative [25, 33], amnestic, and ataxic [34] effects of agonists. On the other hand, anxiolytic and myorelaxant activities rather depend upon the α_2 or α_3 subunits [25, 35], and anticonvulsant effects seem to be depend on the interplay of several subunits amongst which the α_1 is involved in a synergistic manner with the α_3 [36]. These findings could account for the relative absence of myorelaxant and anticonvulsant effects of zolpidem [37]. The relative low potential for dependence and abuse of zolpidem as well as its reduced probability of withdrawal effects compared to benzodiazepine hypnotics has been attributed to its relative α_1 specificity. It was shown that drugs acting through α_1 -GABA_A receptors require higher dose to train [38–40] and that α_1 binding was sufficient but not necessary to produce drug discrimination [41]. A mice study suggested that drug acting at the GABA_A receptor is less likely to lead to physical dependence if there is a combination of binding selectivity and low intrinsic efficacy [42]. Noteworthy, in mice, the deletion of the α_1 subunit or its antagonism reduces the effects of ethanol [43, 44]. Overall, the potential for drug dependence is low but undoubtedly exists on the basis of animal research.

The α_1 -GABA_A receptor subtype accounts for approximately 60% of all GABA_A receptors [45]. Its role in sleep regulation is largely unknown. For instance, knock-out studies in mice have shown that although the sedative action of zolpidem, as defined by a decrease in locomotor activity, was absent in α_1 (H101R) mice, its sleep EEG effects (reduction of rapid eye movement –REM– sleep and increase in non-REM sleep) was largely unchanged [46]. In contrast, in wild-type mice, zolpidem markedly reduced non-REM EEG power in a broad frequency band >5 or >9 Hz, depending on the dose, whereas in α_1 (H101R) mice, the power reduction was either absent or limited, again depending on the dose [46]. These findings suggest that the sleep-promoting effect of zolpidem is mediated by a different mechanism than is its effect on the duration of sleep stages. Low frequency synchronized EEG, including spindle and delta waves that characterized non-REM sleep are thought to arise as a result of discharge interaction between GABAergic thalamic nucleus reticularis neurons and thalamocortical relay neurons [47]. Zolpidem could interfere with these electrophysiological mechanisms because thalamocortical neurons express α_1 -GABA_A receptor subtype [48]. Transitions

from wake to sleep appear to be triggered by sleep active neurons located in the preoptic area of the anterior hypothalamus [49, 50]. Since these neurons are GABAergic and project to all other arousal system, they appear to underlie the somnogenic effects of indirect GABA_A agonists such as benzodiazepine and zolpidem [49]. For instance, histaminergic neurons belonging to the tuberomammillary nucleus (TMN), one of the key wake-promoting structures [51, 52], contain $\alpha 1$ -GABA_A subunits [53]. There are some evidences that zolpidem could inhibit TMN neurons because c-Fos expression in the TMN is completely suppressed after systemic administration of GABA_A modulator acting through the benzodiazepine binding site [54].

3 Pharmacokinetic and Metabolic Profiles

The pharmacokinetic and metabolic profiles of zolpidem have been comprehensively reviewed elsewhere [55–58] and the present section provides a brief overview of the human pharmacokinetics of zolpidem. Of particular note, data presented below relate to young or elderly healthy subjects and to special population such as patients with renal or hepatic insufficiency.

Briefly, the drug is readily absorbed from the gastrointestinal tract. Its limited first-pass hepatic metabolism results in an oral bioavailability of 65–70%. Repeated administration of zolpidem does not modify its absorption rate, and the bioavailability of the compound is not modified in elderly patients [59]. After a single dose of 10 mg zolpidem-IR to healthy subjects, peak plasma concentrations (T_{\max}) are attained after about 50 min [range 30–120 min] and the mean terminal half-life ($T_{1/2}$) is about 2.5 h [60, 61]. As expected, the pharmacokinetic profile of the extended-release formulation (zolpidem-ER) differs from the original immediate release-formulation (zolpidem-IR) (Fig. 2). Zolpidem-ER is a two-layer tablet that provides biphasic release of zolpidem: a first layer dissolves immediately and releases 60% of the drug content and a second layer erodes slowly and releases the remaining 40% of the drug. A study comparing zolpidem-ER 12.5 mg with zolpidem-IR 10 mg in healthy male volunteers found that zolpidem-ER produced slightly longer T_{\max} (90 min; range 30–230 min), lower plasma peak concentration (C_{\max} ; 134 versus 167 ng/ml), and higher area under the concentration curve value (AUC; 740 versus 589 ng·h/ml) [62]. Due to its short $T_{1/2}$, zolpidem does not accumulate in chronic administration, since the drug is no longer present in the body at the time of the next administration.

Zolpidem should not be administered with or immediately after a meal given that food delays its absorption; food-effect studies in healthy volunteers demonstrate that food decreases AUC of IR-zolpidem by 15% and of ER-zolpidem by 23% and C_{\max} by 25% for IR and 30% for CR, while T_{\max} was increased by 50% (IR) or 60% (ER). However, the half-life remains unchanged [63]. About 92% of the drug is bound to plasma proteins, a fraction that was found independent of concentration between 40 and 790 ng/mL [60]. Consequently, the direct excretion of zolpidem is

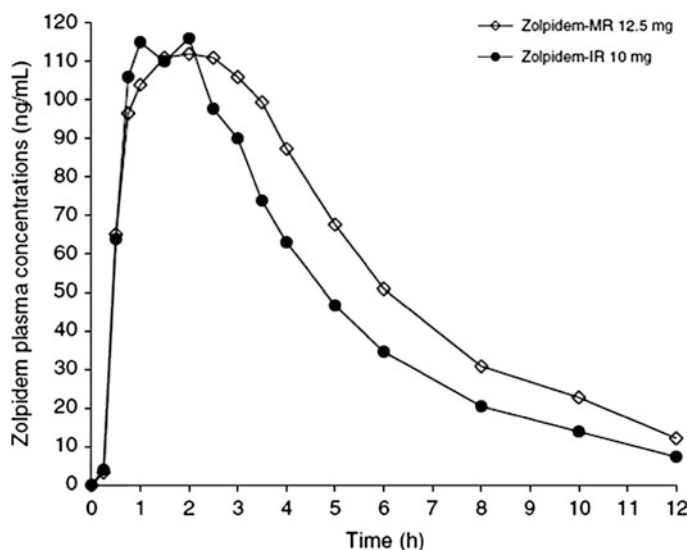


Fig. 2 Pharmacokinetics of zolpidem controlled-release (CR) versus original zolpidem. Mean plasma zolpidem concentrations over time following a single oral dose of zolpidem CR 12.5 mg or original zolpidem 10 mg are shown (reproduced with permission of Weinling et al. [62])

negligible and its clearance is mainly a metabolic one. Zolpidem is extensively metabolized in three main inactive metabolites, and the unchanged compound is observed only in trace amounts in urines [64]. The biotransformation in humans principally involves CYP3A4 with a minor contribution of the CYP1A2 and CYP2C9 isoforms of cytochrome P450 [65]. Besides the involvement of different isoforms of the cytochrome P450, the good bioavailability of zolpidem ($F = 70\%$) limits the eventual metabolic drug interaction since only 30% of the dose will be metabolized [64]. However, coadministration with rifampicin (a CYP3A4 inducer) results in significant decreases in the AUC, C_{\max} , and $T_{1/2}$ values of zolpidem; on the opposite, the CYP3A4 inhibitor itraconazole has been shown to increase zolpidem AUC [56]. Clinically important drug interactions with zolpidem have been extensively reviewed elsewhere [55, 65]. As with any hypnotic drug, pharmacodynamic interactions occur if zolpidem is coadministered with a CNS depressant drugs requiring dosage adjustment of zolpidem. Alcohol also increases the zolpidem-induced sedation and lowering of psychomotor performance [64].

The pharmacokinetic of zolpidem does not appear to be affected to a clinically significant extent by smoking status, ethnicity, sex, or use of oral contraceptives [55, 66, 67]. The kinetic profile of zolpidem has been studied in special population such as elderly subjects or patients with hepatic or renal impairment. In a study comparing young (21–42 years) to older volunteers (66–85 years), C_{\max} and AUC were significantly increased whereas oral clearance was significantly reduced in the older group [68]. Accordingly, in elderly patients, the recommended dosage is 5 mg/day for zolpidem-IR and 6.25 mg/day for zolpidem-ER. The following

pharmacokinetic parameters were observed for healthy subjects older than 65 years after single administration of 5 mg zolpidem-IR [68] and 6,25 mg zolpidem-ER [63]: T_{\max} about 60 min with IR and 120 min with CR, C_{\max} about 100 ng/ml with IR and 70,6 ng/ml with CR, AUC about 400 ng*h/ml with IR and 413 ng*h/ml with CR, $T_{1/2}$ 2.5 h with IR and 2.9 h with CR. In patients with hepatic insufficiency, biphasic elimination could be observed with a $T_{1/2}$ of 9.9 h; therefore, dosing should be modified in these patients and zolpidem initiated at the lowest dosage [64]. Zolpidem pharmacokinetics are not significantly different in patients with end-stage renal failure. Therefore, no dosage adjustment is deemed necessary in these patients [64].

New delivery forms of zolpidem aimed at bypassing the gastrointestinal tract and the liver through direct oral mucosa absorption have recently been developed (ZolpimistTM, EdluarTM, and IntermezzoTM). These new formulations should demonstrate different zolpidem pharmacokinetic profiles than those produced by a swallowed tablet, and the same therapeutic benefits should be achieved more quickly. Indeed, the initial bioavailability at 5, 10, and 20-min of sublingual IntermezzoTM 3.5 mg was found higher than those of AmbienTM 10 mg [69]. EdluarTM sublingual tablet is described as being bioequivalent to AmbienTM tablet with respect to C_{\max} , AUC, and $T_{1/2}$ and having a pharmacokinetic profile characterized by rapid absorption with a T_{\max} comparable to those of AmbienTM [70]. Published pharmacokinetic parameters of the oral spray ZolpimistTM were also similar to those of AmbienTM [71].

4 Therapeutic Efficacy

The sleep-inducing effect of zolpidem has been convincingly demonstrated in a variety of population: healthy subjects, poor sleepers, nonelderly and elderly patients with chronic primary insomnia, and patients with secondary or comorbid insomnia. Efficacy studies have been extensively reviewed recently by Monti and colleagues [58, 72]. This section focuses on results from well-designed placebo-controlled clinical trials in chronic and/or primary insomnia. Subjective (questionnaire) and objective (polysomnography) sleep parameters were used as efficacy endpoints such as sleep latency, wake after sleep onset (WASO), number of awakening (NAW), total sleep time (TST), sleep efficiency, sleep quality, or daytime functioning.

4.1 Efficacy Studies Performed with Zolpidem-IR in Nonelderly Insomniacs

With one exception [73], zolpidem efficacy studies in nonelderly insomniacs were not performed on DSM defined primary insomniac patients but rather on patients

diagnosed according to ICSD criteria [74], or more generally, to study defined diagnostic criteria [75–79], one of those being that their symptoms last for at least 1 month. Other methodological shortcomings are that some of these studies were underpowered in terms of sample size [73, 74, 76]. Three studies [73, 75, 76] used screening polysomnography recordings in order to include patients on basis of objective sleep initiation and/or maintenance disturbances. These studies investigate a 2-week [73], a 4-week [76], or a 5-week [75] administration of zolpidem-IR 10 mg [73, 75, 76] and 15 mg [75]. Results showed that zolpidem decreases latency to stage 2 sleep [75] and WASO [76] and improves TST [76] and sleep efficiency [73, 75]. Regarding sleep architecture, one study found that zolpidem increases stage 2 sleep [76], and another that it decreases REM sleep [75]. In double-blind placebo-controlled studies, subjective sleep was found improved in terms of sleep latency [73, 75], NAW [75], TST [75] and sleep quality [75, 76]. In larger samples of patients with chronic insomnia, the effect of zolpidem on subjective sleep was more consistent and robust. These studies investigated a 1–3 weeks treatment of zolpidem-IR 10 mg [77–79], 15 mg [79], or 20 mg [77] and could demonstrate significant improvement in most if not all parameters including sleep latency, WASO, NAW, TST, sleep efficiency, and sleep quality.

4.2 Efficacy Studies Performed with Zolpidem-IR in Elderly Insomniacs

The three polysomnographic studies that investigated the effects of zolpidem in elderly insomniac subjects have been recently reviewed elsewhere [80]. Only one of these studies was appropriately designed and investigated the effects of zolpidem-IR (10 mg) versus brotizolam (0.25 mg) during three consecutive nights in 14 nonorganic insomniac patients (age: 54.9 ± 8.9 years) using a double-blind cross-over design [81]. Results show that, during the 150-min period following T_{\max} , zolpidem showed significant stage 2 sleep-inducing effect during the 1st and the 2nd treatment nights. Noteworthy, the effects of zolpidem significantly differentiate from those of brotizolam on slow wave sleep (greater with zolpidem during the T_{\max} periods of the 1st and the 3rd nights) and on REM sleep (lower with zolpidem during the 3rd whole-night recording). Unfortunately, the drug effects on latency to sleep stage 2, TST, and WASO were not reported. Subjective sleep studies performed in elderly insomniacs were single-blind designed, with one exception that was reported as an abstract form [82]. Results grossly replicate those obtained in younger insomniacs and show that compared to placebo, zolpidem-IR 5 mg decreases NAW and increases TST and sleep quality in 50 DSM-IV primary insomnia outpatients with a mean age of 74 years. Sleep latency was however not affected. A single-blind study [83] in a large sample of ICSD psychophysiological insomniacs ($n = 769$) with a mean age of 72.9 years showed that zolpidem-IR 5 mg decreases sleep latency and NAW and increases TST and quality of sleep.

4.3 Comparative Studies Performed with Zolpidem-IR and Other Hypnotic Drugs

About 13 studies compared zolpidem-IR to other hypnotic drugs in patients with insomnia; these studies have been extensively reviewed elsewhere [56, 72]. Since most of these studies did not specify primary endpoints or predefined between-drug equivalence criteria [72], statistical results showing no between-drug difference are not easily interpreted. Consequently, the present section will mostly focus on the five placebo-controlled trials. Three of the placebo-controlled studies include patients aged 18–65 years and compared zolpidem-IR 10 mg to trazodone 50 mg (a 2-week study in 306 patients [84]), doxylamine 15 mg (a 2-week study in 338 patients [85]), or triazolam 0.5 mg (a 4-week study in 24 patients [86]), while the two others compared zolpidem-IR 5 mg versus zaleplon 5 and 10 mg (a 2-week study in 549 patients [87]) or triazolam 0.125 mg and temazepam 15 mg (a 4-week study in 335 patients [88]) in elderly insomniacs. DSM criteria were used to recruit primary insomniacs in one study [87] whereas others used study-defined criteria. The all five trials used subjective sleep to assess efficacy with additional polysomnography parameters in one study [86]. On the whole, these studies show that zolpidem and comparative drugs were more effective than placebo in terms of sleep latency [85–88], TST [84–88], NAW [84–86], WASO [84–86], and sleep quality [84–86]. Some between treatment differences were however observed (1) sleep onset latency was significantly shorter with zolpidem-IR 10 mg than with trazodone 50 mg [84] and with zolpidem-IR 5 mg than with zaleplon 5 mg [87] or triazolam 0.125 mg [88], (2) the effects on TST, NAW, and sleep quality were more pronounced with zolpidem-IR 5 mg than with both zaleplon 5 and 10 mg [87], and (3) compared to placebo, zolpidem-IR 10 mg decreased objective total wake time whereas triazolam 0.5 mg augmented REM latency [86].

4.4 Intermittent and “As-Needed” Studies with Zolpidem-IR

The favorable pharmacokinetic and safety profiles of zolpidem (short half-life and low risk for rebound insomnia, see Sects. 3 and 5.3) and concerns regarding the potential dependence of the long-term use of hypnotics has led to investigations on the intermittent (nonnightly) or “as-needed” use of zolpidem. Cluydts et al. [89] initiated a series of trial in that field by comparing in 160 patients with chronic insomnia a 2-week treatment of zolpidem-IR 10 mg given either continuously or intermittently (each week patients received zolpidem for the first 5 nights, followed by placebo for 2 nights) in a double-blind parallel group design. This pilot trial did find a statistical trend ($p = 0.06$) for equivalence in efficacy of the continuous versus intermittent regimens [89]. A second 2-week trial was adequately powered and included 795 chronic insomnia patients [90]; in that study, one week of the intermittent regimen consists of zolpidem-IR 10 mg in the first 2 nights followed by

a random allocation to zolpidem or placebo (three zolpidem and two placebo nights, with placebo administered in nonconsecutive nights). Proportion of patients rated as “much” or “very much” on the CGI-I score was 65.2% in the continuous group and 58.6% in the intermittent group, a result that failed to demonstrate the noninferiority of the intermittent use [90].

The intermittent use trial design did probably not reflect usual clinical practice scenario where insomniac patients are prone to take hypnotics on an “as-needed” basis. Therefore, three double-blind parallel group trials investigated whether “as needed” use of zolpidem-IR 10 mg was superior to “as needed” use of matched placebo in DSM-IV primary insomniacs. Treatment regimens were no fewer than three and no more than five tablets to be taken during 8 weeks (163 patients [91]) or 12 weeks (199 patients [92]), or ten tablets a week during 3 weeks with the instruction to take as few tablets as possible (245 patients [93]). The three studies showed a significant effect of zolpidem-IR 10 mg according to clinician or patient global rating scales but mixed results on self-reported subjective sleep. For instance, TST was improved in two trials [91, 92], sleep quality in the other [93], and sleep parameters such as sleep latency [92], WASO [92], or NAW [91, 92] were inconsistently improved. It has been suggested that zolpidem efficacy could have been masked by the fact that patients would have improved their sleep hygiene during the study because they were subject to protocol rules [91]. Another point is that these results include both pill and nonpill nights.

4.5 Studies Combining Zolpidem-IR to Cognitive Behavioral Therapy

Two studies evaluated the added value of combining zolpidem to cognitive behavioral therapy (CBT) for the treatment of insomnia. In a randomized, placebo-controlled study of 63 DSM primary insomniacs aged 25–64 years suffering of chronic sleep onset insomnia, Jacobs et al. [94] could not demonstrate additional benefits of the combination of zolpidem to CBT. Pharmacotherapy first consisted of a 4-week zolpidem-IR 10 mg administration followed by a 2-week zolpidem-IR 5 mg period where the drug was administered every night on the first week and every 2 nights on the second week. Patients who received CBT attended four 30-min treatment sessions and had one telephone treatment session over 6 weeks. Pharmacotherapy and placebo were administered in a standard double-blind fashion, whereas the combination condition received active medication. Accordingly, four treatment groups were constituted: placebo, zolpidem, CBT alone, and CBT combined with zolpidem. Subjective outcome measures were sleep latency (primary endpoint), sleep efficiency, and TST assessed with 2-week sleep diaries (before treatment, during the 3rd and the 4th treatment week, and after treatment). Patients were also asked to fulfill 1-week sleep diaries 1, 3, 6, and 12 months after treatment in order to ascertain if the treatment benefits are maintained. Objective sleep was assessed with a home-based sleep monitoring device during three

consecutive nights before and at the end of the treatment procedures. Results showed that the most effective intervention in terms of sleep latency and sleep efficiency was CBT whereas zolpidem alone produced only moderate benefit and did not increase CBT efficacy. None of the treatment was effective on TST or on objective sleep parameters. Long-term follow-up data available only for the CBT and the CBT-zolpidem combined groups indicate a sustained treatment effect on sleep latency and sleep efficiency in both groups [95].

The other study investigated the short-term and long-term effects of CBT, singly and combined with medication, in 160 patients aged 50.3 ± 10.1 years suffering from DSM primary insomnia of at least 6-month duration [94]. Subjective sleep (derived from sleep diaries) and objective sleep (derived from polysomnographic recordings) were assessed at baseline, at the end of an initial 6-week treatment period, at the end of an extended 6-month treatment period, as well as 6 months later during a follow-up visit (only subjective sleep). The initial treatment consisted of either CBT alone or CBT combined with nightly administration of zolpidem-IR 10 mg. Results showed that both treatments improved most subjective and objective sleep parameters (sleep latency, WASO, and sleep efficiency). Objective TST was significantly decreased in both treatment groups reflecting the fact that CBT typically involves restricting time spent in bed; however, subjectively, patients with the combined treatment were estimated having a prolonged TST, and the difference between CBT alone and CBT combined with zolpidem (16 min) was statistically significant [94].

After completing this 6-week initial treatment, patients were randomized a second time to an extended 6-month treatment in four groups; patients initially treated with CBT alone for 6 weeks were randomized to either extended CBT for 6 months (group 1) or no additional treatment (group 2), whereas patients who were treated with CBT combined with zolpidem during the first weeks were randomized either to extended CBT alone (group 3) or to extended CBT combined with an “as-needed” use of zolpidem-IR 10 mg (group 4). Initial treatment effects were generally maintained and subjective TST was even found improved in groups 1, 2, and 3. However, in groups 1, 3, and 4, some subjective (WASO in group 4) and objective (sleep latency in group 1, WASO in groups 3 and 4) parameters worsened. Sleep improvements achieved after the extended 6-month treatment were well maintained over time since there were no further changes for any variable except for subjective TST, which was further increased in group 2 [94].

A tentative conclusion that follows from these two studies would be that extended CBT does not add significant benefit to an initial 6-week CBT treatment and that the addition of zolpidem to CBT produced some added benefits to outcome when it is limited to the initial treatment phase.

4.6 Studies with New Formulations of Zolpidem

Zolpidem-ER has been the subject of several efficacy studies. The drug has been designed to both help the patient to fall asleep (the rapidly dissolving layer of the

tablet) and to maintain sleep (the slowly dissolving layer of the tablet). The differential effect of the two formulations on sleep maintenance is illustrated by a study in a model of middle-of-the-night insomnia [96]. In this single-dose 9-way cross-over study, 54 healthy subjects were awakened for 30 min 2.5, 3.5, or 4.5 h postdose on three separate nights. Upon returning to bed, subjects were exposed to a noise stimulus that is known to prolong latency to persistent sleep (LPS). Results show that the two formulations were effective in reducing LPS compared to placebo and that they not differ at 3 h postdose; however, a significant greater improvement on LPS was observed at 4 and 5 h postdose with zolpidem-ER 12.5 mg than with zolpidem-IR 10 mg [96]. These results are corroborated by another ER/IR comparison showing in a daytime study that the pharmacodynamic effects on beta EEG power were greater for zolpidem-ER 12.5 mg compared to zolpidem-IR 10 mg [61].

At the present time, results of three efficacy studies of zolpidem ER in DSM primary insomnia have been published. The first study [97] randomized 212 patients aged 44.3 ± 3.0 years in a double-blind, placebo-controlled, parallel-group 3-week study with polysomnographic recordings on nights 1 and 2 and 15 and 16 of the treatment period. Only patients having objective sleep maintenance difficulties evidenced during two screening nights were included in the study. Results revealed significant improvement in objective sleep initiation and maintenance parameters (LPS, WASO during the first 6 h of sleep, NAW, and SEI) both at the beginning and after 2 weeks of double-blind treatment [97]. The second study used a comparable design and extended these results to elderly patients; it documents the effects of a 3-week treatment of zolpidem-ER 6.25 mg in 205 patients aged 70.2 ± 4.5 years [98]. At the beginning (nights 1 and 2) and after 2 weeks of treatment (nights 15 and 16), significant objective improvements were observed with zolpidem, in particular with respect to LPS and WASO that were corroborated with subjective assessments [98]. A long-term study investigated the efficacy of a 6-month administration of zolpidem-ER 12.5 mg in DSM primary insomniac patients. Patients ($n = 1,018$ aged 45.7 ± 11 years) were randomized according to a double-blind placebo-controlled parallel group design. Treatment was administered on an "as needed" basis, patients being instructed to take no more than one tablet per night for a minimum of three tablets and a maximum of seven tablets per week. Results show very consistent improvement in subjective sleep, i.e., that zolpidem was more effective than placebo in terms of sleep latency, TST, NAW, WASO, and sleep quality at every time-point [99].

Some efficacy studies with the new transmucosal formulation of zolpidem have recently been published. As expected, results of these studies suggest that these formulations provide a more rapid onset of action than those produced by a swallowed tablet. For instance, two studies indicate that sublingual EdluarTM tablets display stronger sleep initiation effects than those of oral zolpidem and comparable effects in terms of sleep maintenance [100, 101]. The first study was performed in 21 healthy volunteers in a postnap model of insomnia and showed that a single administration of the 10 mg sublingual formulation could reduce LPS by about 30% compared to the oral formulation (Fig. 3) and has comparable efficacy on sleep maintenance parameter [100].

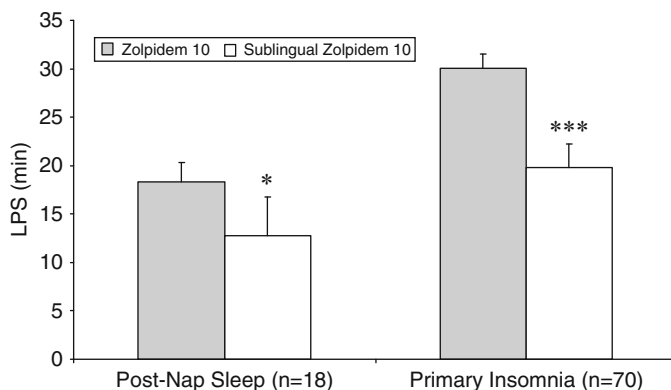


Fig. 3 Effects on latency to persistent sleep (LPS) of 10 mg zolpidem administered as an oral (Ambien™) or as a sublingual (Edluar™) formulation in a postnap model of insomnia in 18 healthy subjects and in 70 patients with primary insomnia (mean + SEM; * $p < 0.05$; *** $p < 0.001$)

These results were confirmed in a randomized double-blind crossover multicenter study that includes 70 DSM primary insomniac patients aged 19–64 years and selected on basis of polysomnographic criteria of sleep onset insomnia. Treatment was administered once, when polysomnographic recording started, using a double-dummy design. Edluar™ 10 mg shortened LPS with 34% or 10.3 min as compared to Ambien™ 10 mg (95% CI: -4.3 to 16.2 min, $p < 0.001$) (Fig. 3). Moreover, the two formulations were comparable in terms of TST, and the improvement in subjective sleep did not differ between the two treatments [101]. Intermezzo™ is a low-dose sublingual formulation of zolpidem developed to be administered following middle of the night (MOTN) awakenings in patients with sleep maintenance difficulties. Its efficacy has been evaluated in 82 patients aged 45.9 ± 12 years with a DSM diagnosis of primary insomnia using a double-blind placebo-controlled crossover study [102]. Patients were awakened 4 h after lights out, dosed with sublingual zolpidem (1.75 or 3.5 mg) or placebo, kept awake for 30 min, and then returned to bed for an additional 4 h. Results demonstrated that MOTN dosing of sublingual zolpidem significantly improve LPS and TST as well as sleep quality and level of refreshed sleep with a dose–effect relationship [102].

5 Safety and Adverse Effects

The next sections first briefly summarize zolpidem tolerability data and then review daytime residual effects, memory and psychomotor outcomes, rebound insomnia, and abuse/dependence potential, which are also relevant to the safety profile of a hypnotic drug.

5.1 *General Tolerance*

A first overview of the safety profile of zolpidem published in 1988 was based on data from 23 clinical trials undertaken during the drug's development [103]. The most serious adverse events reported were confusions and falls, principally in those aged 75 or over at a dose of 20 mg. The most common side effects at the 10 mg dose were drowsiness, headache, nausea, vertigo, and amnesia occurring in 2–4% of all patients [103]. This safety profile was in large part confirmed in subsequent reviews [56, 104, 105] and in postmarketing studies showing that treatment-emergent adverse events occur in about 2% of zolpidem recipients (i.e., 629 of 30,043), the most frequently reported being dizziness, hypotension, headache, and nausea each occurring in about 0.3–0.5% of patients [106]. Elderly patients receiving zolpidem-IR 5 mg reported the same nature of adverse events than those reported by adult patients receiving the 10 mg dose [56]. Studies with zolpidem-ER 12.5 and 6.25 mg in adult and elderly patients, respectively, showed that the most commonly observed adverse events were somnolence, headache, and dizziness [97–99]. The adverse event profile of sublingual formulations of zolpidem was comparable to those of the oral formulations [100, 101].

Some rare and anecdotal drug effects have to be further discussed here. For instance, rare cases of presumably anaphylactic reaction have been observed and probably reflect hypersensitivity to the drug itself or to other ingredients of the tablet [63]. Several cases of delirium, nightmares, and hallucinations have been described. Although nightmares seem to occur idiosyncratically, hallucinations and delirium could be dose-dependent [107]. In particular, hallucinations have been described when zolpidem was coadministered with fluoxetine [108] or fluvoxamine [109], two CYP3A4 inhibitors that could potentially enhance blood levels of zolpidem. However, other authors suggest that a pharmacodynamic interaction between serotonin reuptake inhibition and zolpidem may lead to hallucinations in susceptible individuals [110]. More recently, case reports describing various parasomnia-like behavioral changes occurring after zolpidem intake have been published. It includes sleep-related complex behavior such as sleepwalking, sleep conversation, sleep eating, sleep sex, sleep driving, and sleep shopping, frequently with the amnesia of the event [111]. There are some indications for a higher probability of behavioral changes with zolpidem-ER than with zolpidem IR [112]. Drug-induced sleep-related complex behavior is not a peculiarity of zolpidem and has been described with other Z drugs and more generally with GABAergic acting compounds [111].

5.2 *Next-Day Residual Effects*

As with many other hypnotic drugs, the severity of next-day residual effects with zolpidem appears to be dose-related and not significantly different from those observed with other hypnotics in postsleep efficacy assessments [56].

Therefore, this subsection reviews data coming from studies that assessed residual effects of recommended dosage of zolpidem with standardized tests and focuses on patient studies rather than on studies performed in healthy subject. The latter issue is of importance since many reports show that patients with insomnia have electrophysiological and psychomotor evidences of increased CNS arousal level [113, 114] as well as signs of homeostatic dysregulation [115]. These disturbances would explain the lack of excessive daytime sleepiness generally encountered in primary insomniac patients despite their chronic sleep debt [116–118]. Moreover, it questions whether next-day residual effects of hypnotics observed in healthy controls could be generalized to insomniac patients.

In this context, one may emphasize the fact that patients with insomnia who received zolpidem-IR 10 mg at bedtime did not experience next-day sedation or impairment of psychomotor function compared with patients receiving placebo [119, 120], including for driving performance in a simulator [121] or on the road [122]. In contrast, recipients of flurazepam 30 mg [119], flunitrazepam 1 mg [120], or zopiclone 7.5 mg [121] experienced significant next-day impairment of psychomotor function or driving performance. Another driving simulator study in insomniac patients could not evidence major differences (except for a greater lane position deviation with zolpidem-IR 10 mg compared to temazepam 20 mg and placebo) in psychomotor performances 5.5 h after drug administration, but it appeared that certain patients were more susceptible than others to the drug effects [123]. However, in healthy subjects, residual effects of zolpidem are still apparent on psychomotor objective assessments up to 5 h after nocturnal administration [124]. Thus, although driving the morning following bedtime administration of the recommended dose of zolpidem seems safe, middle of the night administration of zolpidem is not recommended. Another issue regarding next-day residual effects in insomniac patients taking zolpidem is the potential pharmacodynamic interaction with melatoninergic agents. Indeed, in a study in healthy subjects aged 55 years or older, it was shown that the psychomotor and driving performance impairment observed 1 and 4 h after zolpidem-IR 10 mg administration were exacerbated when 2 mg of a controlled-released form of melatonin was coadministered (Fig. 4) [125].

Studies performed in insomniac patients indicate that zolpidem-ER seems to be devoid from significant next-day residual effects on cognitive and psychomotor performance as assessed subjectively [99] or with standardized test [97, 98]. In the two studies performed in insomniac patients, next-day residual effects of the sublingual formulations of zolpidem were shown to be comparable to those of the oral formulations [101] or of placebo [102]. However, the FDA recently denied the approval of the low-dose sublingual formulation *Intermezzo*TM for MOTN insomnia and requested additional data demonstrating that a middle of the night intake of the drug would not present an unacceptable risk of residual effects, with particular reference to next day driving ability.

Daytime studies have shown that memory dysfunctions induced by zolpidem are clearly observed at or near the time of plasma peak concentration and persists for few hours after dosing [126, 127]. Accordingly, next-day memory function is not impaired with zolpidem as stated before. However, little is known about the effects

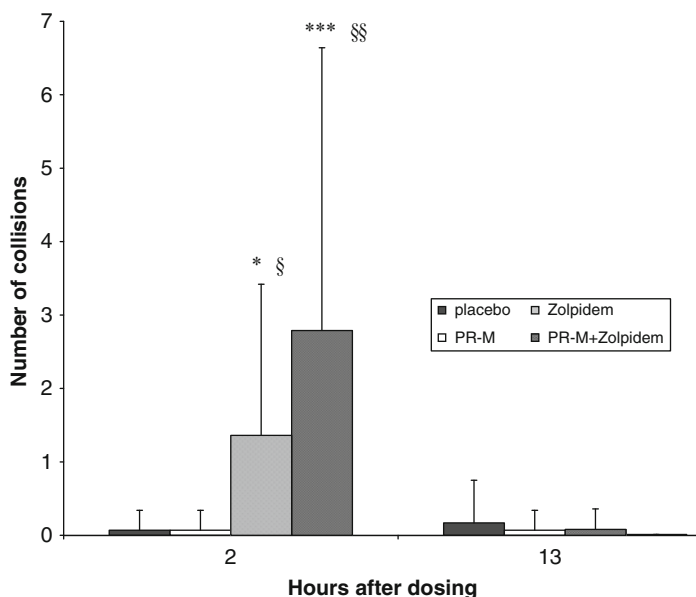


Fig. 4 Number of collisions (against other vehicle or crash barriers) in a simulated driving task of 1 h as a function of the treatment and the hours postdosing [mean + standard deviation; * $p < 0.05$; *** $p < 0.001$ compared to placebo; § $p < 0.05$; §§ $p < 0.001$ compared to PR-M (controlled-released form of melatonin)]

of zolpidem on sleep-related memory consolidation. The two available published studies were performed in healthy subjects. A first study indicates that zolpidem-IR 10 mg (as triazolam 0.25 mg) did not interfere with the nighttime consolidation of declarative memory [128] (free-recall test of ten standard word and seven nonword lists). In the second study, triazolam 0.375 mg clearly affected sleep-dependent motor skill memory consolidation (a motor sequence task), and similar trends were seen with zolpidem-IR 10 mg [129]. The authors postulate that a drug-induced stage 2 increase (that was statistically significant for triazolam but not for zolpidem) would underlie the learning impairment since procedural motor learning has been shown associated with stage 2 sleep. However, the clinical significance of this finding is uncertain and the results need to be replicated in a sample of patients with insomnia where the effects of hypnotic drugs on sleep architecture have to be balanced with their beneficial effects on sleep continuity.

5.3 Tolerance, Withdrawal Reaction, Abuse, and Dependence Potential

The related phenomena of tolerance, withdrawal, abuse, and dependence are probably the adverse effects of hypnotic drugs, which give rise to greatest concern.

According to several long-term studies, there is little evidence to suggest that prolonged administration of zolpidem induces tolerance (i.e., dose escalation when therapeutic effect is no longer achieved) in insomniac patients [130–132]. Nondaily use studies bring further support to low potential for tolerance with zolpidem-IR 10 mg. For instance, zolpidem consumption did not increase in 4- or 8-week placebo-controlled studies [91, 93], nor in two large 3-week observational studies [133, 134] (zolpidem consumption even decrease in one of them [130]). However, in a 12-week placebo-controlled study [92], an increase in dose was observed and placebo recipients took significantly fewer tablets than zolpidem recipients. At the present time, it is not known if other formulations of zolpidem have a different tolerance profile than zolpidem-IR.

Among withdrawal reactions that result from hypnotic discontinuation, rebound insomnia (i.e., worsening of insomnia symptoms after drug discontinuation compared with pretreatment symptoms) has been the subject of many studies, and those concerning zolpidem-IR have been extensively reviewed elsewhere [56]. A meta-analysis including all sleep laboratory studies published on short-acting hypnotics up to 1997 revealed that triazolam was associated with the more intense rebound insomnia and zolpidem with minimal rebound symptoms [135]. The study showed a nonsignificant difference in TST but a significant prolonged sleep onset latency (+13 min) during the first withdrawal night of zolpidem-IR 10 mg. Three out of the four studies that assessed rebound insomnia as a primary endpoint failed to evidence, in contrast to triazolam 0.25 mg in one study [136], signs of rebound insomnia after 2 or 4 weeks of treatment with zolpidem-IR 10 mg [86, 136, 137]. When available, subjective data for sleep latency, TST, WASO, and NAW were in accordance with polysomnographic findings [136, 137]. Using subjective assessments, a study performed in 79 chronic insomniac patients could evidence rebound insomnia (defined as the presence of a least one worsening of TST or of sleep latency of more than 40% compared to baseline during a one-week observation period) after cessation of a 4-week treatment with zolpidem-IR 10 mg; there were, however, no difference between baseline and withdrawal TST or SOL [138]. In elderly patients, evidences of rebound insomnia have been demonstrated in a 2-week study of subjective sleep [87] with patients taking zolpidem-IR 5 mg having a higher incidence of a sleep parameter worsening (TST or sleep latency) during the first withdrawal night. In two other trials performed in elderly insomniacs, there was minimal [88] or no [139] evidence for rebound insomnia.

Studies on the intermittent use of zolpidem also indicate a low [91], if any [92, 140], potential for rebound insomnia. For instance, during the 12-week trial, there were no significant differences between zolpidem and placebo on no-treatment night in terms of sleep latency, TST, WASO, or NAW [92]. Abrupt treatment discontinuation was also studied with zolpidem-ER with mixed results. After a 3-week treatment with 12.5 mg, polysomnographic recordings evidenced a significant worsening of LPS, sleep efficiency, and WASO during the first but not the second withdrawal night [97]. However, during the 6-month intermittent use studies, rebound insomnia could not be evidenced during no-treatment nights or

at the end of the trial [99]. Studies on withdrawal reaction are not available for the transmucosal formulation of zolpidem.

Another withdrawal reaction to hypnotic discontinuation is withdrawal syndrome that results from drug abuse and dependence, a condition rarely encountered with zolpidem in primary insomniacs. Indeed, a review of reported cases of abuse and dependence revealed that these conditions were very low and that most of these patients had a history of substance abuse and/or concomitant psychiatric illness [141]. It has also to be stressed that withdrawal syndromes were observed in patients that largely exceed the recommended daily dosage of zolpidem (up to 1,200 mg/day with a mean of about 400 mg/day) [141]. However, randomized double-blind trials evidenced withdrawal syndrome following gradual withdrawal in patients treated for at least 3 months with zolpidem-IR 10 mg/day [142]. On the other hand, postmarketing surveillance studies brought either no [130, 143, 144] evidence or infrequent [145] evidence of abuse or dependence of zolpidem. It has however to be stressed that zolpidem has been included (along with other benzodiazepine receptor agonists) in Schedule IV of the Convention on Psychotropic Substances of 1971 [146].

6 Dosage, Administration, and Place in the Management of Insomnia

The 2005 NIH statement regarding treatment of chronic insomnia [10] indicates that the two therapeutic approaches demonstrating well-documented evidence of efficacy in the treatment of primary insomnia are CBT and benzodiazepine receptor agonists. Unfortunately, little is known about the comparative benefits of these treatments or their combination. As far as zolpidem is concerned, two studies indicated no clear-cut difference in efficacy between the two treatments excepting for subjective (but not objective) sleep latency and sleep efficiency that were found more improved with CBT than with zolpidem [95]. Moreover, one study could not demonstrate additional benefits of the combination of zolpidem to a 4-week CBT treatment [95], and the other suggests that zolpidem produced some added benefits to a 6-month CBT outcome when its administration is limited to the initial 6-week treatment phase [94].

However, few clinicians have the expertise of CBT techniques, and pharmacological approaches alone (including zolpidem) are in widespread use for the treatment of primary insomnia [13]. Compared to classical benzodiazepine drugs (such as flurazepam, flunitrazepam, temazepam, or triazolam), zolpidem shares with the other “Z” drugs (zaleplon and zopiclone) its higher selectivity toward α_1 subunits of the GABA_A receptor. Since the α_1 subunit affinity carries some specificity for the sedative effect of benzodiazepines, Z drugs were developed in an attempt to reduce benzodiazepines-related adverse events. Indeed, zolpidem adverse effects are certainly less abundant and less severe than with benzodiazepine

hypnotics despite comparable efficacy in terms of sleep induction. The main differences between Z drugs related to their pharmacokinetic properties: the ultrashort half-life of zaleplon (about 1 h) appears appropriate only for patients with sleep-onset insomnia or following MOTN awakenings in patients with sleep maintenance difficulties [124, 147]. On the other hand, zolpidem and zopiclone with their longer half-life (approximately 2.5 and 5 h, respectively) counteract difficulties both in initiating and maintaining sleep. It however appears in certain zolpidem/cyclopyrrolones comparison studies that the improvement of zolpidem-IR was restricted to the first part of the night and the drug was less effective than eszopiclone 3 mg regarding objective sleep maintenance parameters such as WASO and NAW [148], whereas other studies indicate that next-day residual effects [121] or rebound insomnia were more frequent with zopiclone [149].

Zolpidem-ER provides extended plasma concentration beyond 4 h after administration and has been shown more effective than zolpidem-IR at 4 and 5 h postdose in a model of middle-of-the-night insomnia [96]. Moreover, zolpidem-ER seems to be devoid from significant next-day residual effects in insomniac patients [97–99]. Consequently, the available evidences tend to indicate that zolpidem-ER is an effective and safe hypnotic drug for the treatment of primary insomnia characterized by sleep onset and/or sleep maintenance difficulties. Transmucosal absorption of zolpidem by sublingual formulations or oral spray is supposed to provide a more rapid onset of action than those produced by a swallowed tablet. This has been demonstrated for a sublingual formulation of zolpidem (EdluarTM) that displays stronger sleep initiation and comparable sleep maintenance effects than AmbienTM [100, 101]. Short or middle-term studies (upto 12 weeks) on the intermittent and as needed use of zolpidem-IR indicate that the drug is efficacious on nights it is taken with little concern of rebound insomnia the times the drug is not used [89–93]. Moreover, a 6-month placebo-controlled study with zolpidem-ER administered on an as-needed basis shows very consistent improvement in subjective sleep with a safety profile comparable to zolpidem-IR and a lack of tolerance or dependence to the drug [99].

Oral zolpidem is meanwhile available not only as AmbienTM but also as generics and as Ambien CRTM. Available transmucosal formulations are EdluarTM and ZolpimistTM. Half-dose (6.25 mg in place of 12.5 mg for Ambien CRTM and 5 mg in place of 10 mg for the other formulations) is recommended as a starting dose for elderly patients because aging is associated a reduction in the efficiency of drug hepatic metabolism. Short treatment duration is recommended (<4 weeks) but Ambien CRTM has been approved for long-term use. Precautions for use in special patient population (such as in advanced hepatic disease), warnings, and drug interactions are contained in the manufacturer's prescribing information [63, 70, 71].

At the recommended dosages, zolpidem appears to be well tolerated and devoid of next-day residual effects with the most commonly observed adverse events being nausea, drowsiness, headache, and dizziness. A daily use of a recommended dose of zolpidem for at least 2 week has been inconstantly associated with rebound insomnia when the drug is abruptly discontinued [87, 88, 135, 138]. However, no or infrequent evidence of abuse, dependence, or withdrawal syndrome has been

reported [130, 141–145]. Peculiar cases of delirium, hallucinations, or drug-induced sleep-related complex behavior have been reported with zolpidem, as with zopiclone or zaleplon [111]. The latter adverse effects as well as abuse/dependence concerns were mostly, if not exclusively, observed in patients with a psychiatric comorbid condition such as substance abuse disorder or major depression [110–112, 141]. Accordingly, most patients with a history of alcoholism or drug abuse should not receive GABAergic hypnotics, including zolpidem. Caution is advised in depressive patients especially when zolpidem is added to a serotonin reuptake inhibitor [110] such as fluoxetine [108] or fluvoxamine [109].

7 Conclusions

The data reviewed in the present paper demonstrate that zolpidem, synthesized by Synthélabo Recherche in the early 1980s [14], continues to be a useful therapeutic option in the pharmacological treatment of patients with primary insomnia. The original immediate-release forms as well as the new formulations (extended release and sublingual forms) have been shown to improve sleep in patients with insomnia. When administered according to the manufacturer's prescribing information, zolpidem is well tolerated and associated with minimal rebound insomnia and a low propensity to cause next-day residual effects, drug abuse, or dependence.

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Efficacy and Safety of Zopiclone and Eszopiclone in the Treatment of Primary and Comorbid Insomnia

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Abstract Drug enantiomers with chiral factors exhibit different effects on pharmacological activity, metabolism, and toxicity in the human body, and thus may differ in their pharmacokinetic and pharmacodynamic properties. Many currently used medications in clinical practice are mixtures of enantiomers (racemates), and replacing existing racemates with single isomers has resulted in improved efficacy and/or safety profile of the racemic mixtures. This “chiral switch” allows the existing racemate to be switched to one of its isomers and provides a safer, better-tolerated, and more efficacious alternative medication. Since the introduction and widespread use of asymmetric synthesis and chiral separation technologies, as well as the publication of formal FDA guidelines, which encourage the development of chiral drugs, many pharmaceutical manufacturers have developed single-enantiomer drugs.

Zopiclone [(R,S)-zopiclone] is a hypnotic, which has been available in countries outside the United States (US) for over 20 years, while eszopiclone (S-zopiclone) is the dextrorotatory enantiomer of racemic zopiclone, approved as a hypnotic in the United States in 2004. Both zopiclone and eszopiclone are pyrrolopyrazine derivatives of the cyclopyrrolone class, and are both active at GABA_A receptors, whereas (S)-zopiclone has a relatively higher affinity and accounts for much of the action of racemic zopiclone.

The hypnotic effects of zopiclone and eszopiclone have been demonstrated in numerous clinical trials in healthy subjects and patients with insomnia. Both hypnotics are utilized in the management of insomnia in patients who either experience difficulty initiating sleep or are unable to maintain sleep through the night.

Clinical studies utilizing zopiclone at a dose of 7.5 mg administered nightly have demonstrated the hypnotic’s efficacy in reduced sleep onset latency, increased total sleep time, and a reduction in the number of night-time awakenings. In treating insomnia, 7.5 mg zopiclone nightly is at least effective as the benzodiazepine

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hypnotics, flurazepam, flunitrazepam, nitrazepam, temazepam, triazolam, and midazolam, used at approved therapeutic doses. Trials with zopiclone administered for up to 6 weeks duration as well as EEG analysis of sleep parameters in a 17-week study have revealed no marked tolerance to the effects of the hypnotic. Additionally, studies with zopiclone have demonstrated that it does not have a high dependence potential or cause rebound insomnia, and does not cause significant next-day psychomotor impairment.

In clinical trials, eszopiclone 2 mg and 3 mg in adults with chronic insomnia significantly improved subjective and objective sleep measures including improved sleep efficiency, sleep latency, total sleep time, wake time after sleep onset, number of awakenings, number of nights awakened weekly, and quality and depth of sleep. In elderly patients, eszopiclone 2 mg was associated with significantly shorter sleep latency compared to placebo and significantly improved ratings of sleep quality and depth, wake time after sleep onset, sleep duration, and decreased cumulative number of naps. Long-term treatment of primary insomnia with eszopiclone for 6 months improved daytime functioning and health-related quality of life. In clinical trials of up to 12 months duration, eszopiclone 3 mg showed a sustained beneficial effect on sleep induction and sleep maintenance, was well tolerated, with no clinically significant evidence of tolerance, rebound insomnia or dependence noted. Nightly use of eszopiclone 3 mg did not impair next-day driving-related skills or measures of cognition relative to placebo. Randomized, double-blind trials have demonstrated that eszopiclone 3 mg administered once nightly for 4–8 weeks produced significantly greater improvements in measures of sleep induction and maintenance compared to placebo in patients with insomnia and comorbid conditions, as well as improvements in certain measures of the comorbid conditions compared to standard therapies alone. In 2005, eszopiclone was the first sedative hypnotic approved by the US Food and Drug Administration (FDA) for the long-term management of chronic insomnia.

Both zopiclone and eszopiclone appear to be well tolerated in short-term and in longer-term studies in nonelderly and elderly patients. Numerous studies performed on a self-reporting basis show the absence of serious adverse events. The most commonly reported adverse events in clinical trials with both zopiclone and eszopiclone are unpleasant or bitter taste, headache, dizziness, and dry mouth.

1 Isomerism and Pharmacologic Properties

Stereoisomers are molecules that are identical in atomic constitution and bonding, and hence have the same chemical formula, although they differ in the 3D arrangement of the atoms. Approximately one in every four drugs marketed exists as a combination of isomers, and most of these compounds exist as racemates owing to the presence of a chiral or asymmetric carbon atom. Hence they contain a 50:50 mixture of enantiomeric structures related to each other as nonsuperimposable mirror images. Enantiomers cannot be separated via crystallization, distillation, or

chromatography on columns packed with achiral materials. If the other reactants are achiral, then both enantiomers will possess identical reactivity, and their physical properties (e.g., melting and boiling points) will also be the same. Enantiomers have optical properties and differ in how they rotate plane-polarized light; dextrorotatory compounds rotate light clockwise, while levorotatory compounds rotate light counterclockwise. Hence, when labeling agents with their chemical names, dextrorotatory compounds are designated with the (+) symbol, while the levorotatory compounds are identified with the (−) mark. The Cahn-Ingold-Prelog convention, which assigns a priority to each substituent on the chiral center, by ranking atoms attached to a chiral carbon according to atomic number, may also be used to describe the stereochemistry of an enantiomer. Finally, to describe its stereochemical descriptor, the molecule is visualized in an orientation that places the lowest priority substituent directly behind the chiral center. If the remaining substituents decrease in priority around the chiral center in a clockwise direction, the chiral substance is the R (rectus) enantiomer; however, if they decrease in a counterclockwise fashion, then the substance is an S (sinister) enantiomer [1]. The two systems of nomenclature are mutually exclusive; thus the S enantiomer of one compound may be dextrorotatory, while another compound may have its R enantiomer as dextrorotatory.

Prior to the FDA regulations in 1992, pharmaceuticals were usually marketed as a racemic mixture [2]. When a pharmaceutical manufacturer develops a single enantiomer from a pharmaceutical product already marketed as a racemic mixture, this is referred to as a “chiral switch.” The single enantiomer is given a new generic name, additional patent protection, and the manufacturer markets the new agent as a new product, with a different trade name [3]. The enantiomers of chiral substances, although structurally identical, may have different pharmacokinetic properties (absorption, distribution, biotransformation, and excretion) as well as different pharmacological or toxicological effects that are relevant in clinical psychopharmacology [4]. The presence of a second enantiomer in racemic mixtures may be associated with a potential for unwanted effects such as drug interactions, adverse events, altered metabolic or excretory function, and market withdrawals [5]. The use of stereochemically pure agents is thought to offer advantages via increased potency and selectivity, lower adverse events, improved onset and duration, and decreased potential for drug interactions. Thus chiral switches may provide an improved therapeutic index, since often one of the two enantiomers in a racemic product is active and an enhancement of the toxicity may be linked to this activity, while the other enantiomer is markedly less active or inactive and less toxic [6, 7].

2 Chemical and Physical Properties of Zopiclone and Eszopiclone

Zopiclone was the first compound developed as chemically unrelated to benzodiazepines, yet it exhibits a high affinity of binding to benzodiazepine receptors in the brain [8]. Both zopiclone and eszopiclone, the S(+)-enantiomer of racemic

zopiclone, are nonbenzodiazepine derivatives of the cyclopyrrolone class with no structural similarity to zolpidem, zaleplon, benzodiazepines, or the barbiturates.

Zopiclone is a chiral compound that possesses an asymmetric carbon on the pyrazine group in position 2, which allows for racemic zopiclone to possess two isomers or enantiomers (i.e., (R)- and (S)- enantiomers). Malic acid utilized as the resolving agent has been used to separate racemic zopiclone by crystallization, and two different methods have been used to analytically determine the zopiclone enantiomers by HPLC on chiral stationary phases [9]. An automated coupled achiral–chiral liquid chromatographic method has been used to determine the enantiomers of zopiclone and its two chiral *N*-desmethyl and *N*-oxide metabolites in urine [10]. A new chiral separation technology, biphasic recognition chiral extraction for the racemic separation of zopiclone, has been identified and has great significance for preparative separation of racemic compounds [11]. A rapid, simple, and accurate capillary electrophoretic method was developed for the determination of zopiclone and its synthesis precursor [12]. Following the development of a system of making zopiclone derivative, a patent was assigned to Sepracor, the manufacturer of eszopiclone, in 2008 allowing for the preparation of racemic and stereomerically pure derivatives of zopiclone “and pharmaceutically acceptable prodrugs, salts, solvates, hydrates, and clathrates thereof” [13]. In July 2009, a patent in China was issued for development of a method for transformation of crystal form C of zopiclone to obtain crystal form A of zopiclone. This method of invention is cited as having advantages of good safety, production reliability, as well as a high yield and good quality [14].

Zopiclone exhibits chiral inversion in vivo [15], and virtually all sedative and hypnotic activity of racemic zopiclone is attributable to the dextrorotatory isomer, which is approximately twice as active as the racemate [16]. The levorotatory isomer is both almost inactive and more toxic than racemic zopiclone. In mice, for example, racemic zopiclone possesses a toxicity (LD₅₀) in the region of 850 mg/kg, while the dextrorotatory isomer, S(+)-Zopiclone, has a toxicity in the region of 1.5 g/kg, and the levorotatory isomer, R(–)-Zopiclone, possesses an LD₅₀ between 300 and 900 mg/kg [17].

Eszopiclone, the S(+)-enantiomer of racemic zopiclone, is a single isomer, pyrrolopyrazine derivative of the cyclopyrrolone class, and has the prefix *-es* added to the base name of zopiclone for the dextrorotary form, the S-isomer. The chemical name of eszopiclone is (+)-(5S)-6-(chloropyridine-2-yl)-7-oxo-6,7-dihydro-5H-pyrrolo[3,4-b] pyrazin-5-yl 4-methylpiperazine-1-carboxylate. The chemical name for zopiclone is 6-(5-chloro-2-pyridyl)-6,7-dihydro-7-oxo-5H-pyrrolo[3,4-b]pyrazin-5-yl-4-methylpiperazine-1-carboxylate. Both drugs have the chemical formula C₁₇H₁₇ ClN₆ O₃ and their molecular weight is 388.8 Da. Zopiclone is available as 7.5 mg tablets (containing 3.75 mg of the active stereoisomer) and marketed under various trade names such as Imovane, Zimovane, and Zopinon in Europe and Canada, and as Amoban in Japan. Eszopiclone is currently marketed in the United States under the trade name Lunesta and is available as film-coated tablets in dosages of 1 mg, 2 mg, and 3 mg. Both zopiclone and eszopiclone are white to light yellow crystalline solids, very slightly soluble in water, slightly

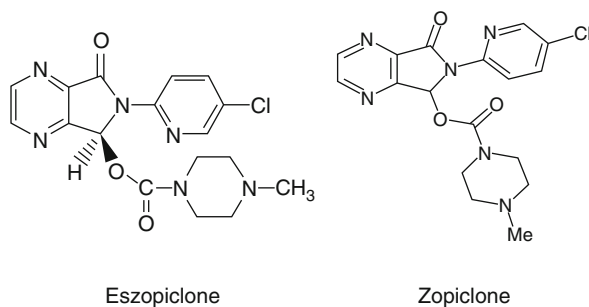


Fig. 1 Chemical structures of eszopiclone, the S(+)-enantiomer of racemic zopiclone, and zopiclone [18, 19]

soluble in ethanol, and soluble in phosphate buffer (pH 3.2). Zopiclone is freely soluble in chloroform and dichloromethane, soluble in dimethyl formamide and 0.1N hydrochloric acid and practically insoluble in acetone. The chemical structures of both eszopiclone and zopiclone are shown in Fig. 1.

3 Mechanism of Action

Zopiclone and eszopiclone's precise mechanism of action in enhancing and promoting sleep is unknown, although their hypnotic effect is thought to result from their interaction with the γ -aminobutyric acid_A (GABA_A) receptors, leading to an increase in chloride transmission that subsequently depresses the central nervous system, slowing brain activity, and promoting sedation. Stereoselective binding at benzodiazepine receptors have been reported and a separate binding site for cyclopyrrolones has been identified. In vitro studies with functional recombinant GABA_A receptors suggest that both racemic zopiclone and eszopiclone bind to α_1 and α_3 subtype receptors on the GABA_A benzodiazepine receptor complex [20]. Zopiclone binds with approximately equal affinity to GABA_A receptors containing different α subunits and more closely resembles a benzodiazepine in terms of its GABA_A receptor binding profile [21]. Thus zopiclone cannot discriminate the behavioral pharmacological effects by selective action on the GABA_A receptors containing α subunits as they specifically relate to the sedating and anxiolytic effects [22]. Eszopiclone's affinity for this receptor complex has been shown to be twice that of its racemic parent, while the levorotatory isomer's affinity for these receptors is negligible [9]. Hence, virtually all sedative and hypnotic activity of racemic zopiclone is produced mainly by the (S)-enantiomer [23]. The half-maximal inhibitory concentration of the dextrorotatory isomer [(S)-zopiclone] has >50-fold higher affinity for the benzodiazepine receptor compared with the levorotatory isomer [(R)-zopiclone] (21 nmol/l and 1,130 nmol/l, respectively) [9]. The binding affinities of (S)-desmethylzopiclone and (S)-zopiclone N-oxide, the two major

metabolites of eszopiclone, to the central benzodiazepine site are >20-fold and >250-fold lower than that of eszopiclone, respectively [24].

Eszopiclone’s interaction with GABA_A α_1 -subunit is less specific than that of zolpidem and zaleplon [23, 25]. A study using site-directed mutagenesis, radioligand binding, and molecular docking compared the structural requirements for zolpidem and eszopiclone binding to α_1 -containing GABA_A receptors. It was noted that unlike eszopiclone, zolpidem relies more on the overall shape of the binding pocket than on specific residue interactions within the benzodiazepine site and may explain why zolpidem is highly α_1 - and γ_2 -subunit-selective [26].

The stereoselective discriminative stimulus effects of zopiclone were recently evaluated in rhesus monkeys [27]. Both the parent drug zopiclone (0.32–17.8 mg/kg) and (S)-zopiclone (0.1–10 mg/kg) substituted for midazolam with similar potencies (>80% of midazolam-appropriate responding). The inactive isomer of zopiclone had a weak substitutive effect even at very high doses (100 mg/kg) and this could be explained by stereoconversion of the compound into the (S)-isomer in vivo. Additionally, (S)-desmethylzopiclone, a metabolite of zopiclone, did not possess any benzodiazepine-like or benzodiazepine antagonist-like discriminative stimulus properties, indicating a novel mechanism of action for the anxiolytic effects of this metabolite other than actions at benzodiazepine receptors.

Zopiclone has been noted to displace benzodiazepines in vitro, due to its greater affinity for benzodiazepine binding sites (α_1 , α_2 , α_3 , and α_5 subunits) in the cerebral cortex, hippocampus, and cerebellum, compared to the benzodiazepines [28]. Zopiclone binding has been shown to involve histidine 101 of the 1-subunit (α_1 H101), which is a necessary component of the benzodiazepine binding site [29]. Compared to the racemate or the R enantiomer, eszopiclone presents higher affinity either to the receptor or to the ion channel (Table 1). Ranking the affinity values (IC₅₀) of the different test compounds for the receptor subtype or for the associated chloride currents, the following results were obtained: zopiclone (IC₅₀ for receptor subtype): $\alpha_1 > \alpha_2 > \alpha_5 > \alpha_3$; zopiclone (IC₅₀ chloride channel current): $\alpha_1 = \alpha_5 > \alpha_3 > \alpha_2$; eszopiclone (IC₅₀ for receptor subtype): $\alpha_5 = \alpha_1 > \alpha_2 > \alpha_3$; eszopiclone (IC₅₀ chloride channel current): $\alpha_5 > \alpha_2 = \alpha_3 > \alpha_1$. Additionally, the IC₅₀ for α_5 -mediated chloride current is of the same magnitude as that for receptor binding at α_5 and α_1 subtypes, suggesting that

Table 1 Selective binding for GABA_A receptor subtypes: human recombinant GABA receptor subtypes in *Xenopus* oocytes [24]

EC ₅₀ (nM) = concentration that resulted in 50% of maximum potentiation of GABA evoked Cl [−] current	Racemic zopiclone	R(−)- zopiclone	S(+)- zopiclone (eszopiclone)	Zolpidem	Zaleplon
$\alpha_1\beta_2\gamma_2$	97	787	240	78	169
$\alpha_2\beta_2\gamma_2$	600	–	99	470	1,360
$\alpha_3\beta_2\gamma_2$	117	>10,000	102	750	2,150
$\alpha_5\beta_2\gamma_2$	97	>10,000	25	–	2,600

– No response or not measurable

the activity of eszopiclone, linked to chloride channel currents, is primarily mediated by α_5 receptor subtype. The same cannot be said for the racemic zopiclone, possibly due to the influence of the R-enantiomer in the results [24]. The GABA subunit α_1 has been implicated in the development of psychological dependence, and further studies suggest that α_1 subunit changes are involved in the development of physical dependence and can be associated with the withdrawal syndrome. Administration of zopiclone for 30 days in mice was associated with no tolerance to GABA_A receptor effects; however, a decrease in GABA_A receptor effects was observed during a month-long administration of benzodiazepines in rats and mice [30].

Based on the EC₅₀ data, racemic zopiclone has its highest potency for α_1 and α_5 receptors with a potency at α_1 that is similar to that of zolpidem, while eszopiclone has its highest potency at α_5 followed by α_2 and α_3 receptors, and is least potent at α_1 . For example, eszopiclone is 3.4 times less potent than zolpidem at α_1 . The differences in the effects of racemic zopiclone and eszopiclone on GABA α -subunits may contribute to the differences noted between the two hypnotics. For example, eszopiclone may have fewer detrimental effects on memory compared with zopiclone, related to the lower efficacy at the α_5 and α_1 subunits, relative to the other units; may have greater anxiolytic and antidepressive effects compared with zopiclone related to the relative modulation of α_2 and α_3 receptor subunits; and may have beneficial effects on comorbid pain related to the modulation of α_2 and α_3 receptors compared with zopiclone [24].

Benzodiazepines are thought to exert sedative and anticonvulsant effects by affecting α_1 - GABA_A receptors and anxiolytic effects by affecting α_2 - GABA_A receptors. Neither racemic zopiclone nor its metabolite (S)-desmethylzopiclone are selective for α_2 - GABA_A receptors; they are active at α_1 -, α_2 -, and α_3 - GABA_A receptors. Thus, it would be expected that both agents would produce sedation and have anticonvulsant and anxiolytic properties. Racemic zopiclone exhibits these properties; however, (S)-desmethylzopiclone does not produce sedation, except at doses that are 50–100-fold higher than that required for anxiolytic or anticonvulsant effects [23]. Like benzodiazepines, zopiclone and its metabolite (S)-desmethylzopiclone inhibit γ -1-bearing GABA_A receptors, N-methyl-D-aspartate receptors (NR1/2B subtype) and nicotinic acetylcholine receptors ($\alpha 3\beta 4$ subtype), which may be associated with the addictive properties of these medications. The metabolite of zopiclone, (S)-desmethylzopiclone, has benzodiazepine-like actions at γ_2 -bearing subtypes of the GABA_A receptor [31].

Recently, eszopiclone via positron emission tomography (PET) has shown to increase the decline in metabolic rate in the pontine and midbrain reticular-activating system in the transition from waking to nonrapid eye movement (NREM) sleep compared to placebo [32]. Following eszopiclone treatment, these and other portions of the arousal systems have lower metabolic rates during sleep. This may represent a drug-induced decrease in the hyperarousal, which has been observed in insomniacs.

4 Pharmacokinetics of Zopiclone and Eszopiclone

The pharmacokinetic parameters of zopiclone and eszopiclone have been studied in healthy adult and elderly volunteers and patients with hepatic or renal impairment. Selected pharmacokinetic parameters of racemic zopiclone, R(–)-zopiclone, and S(+)-zopiclone are summarized in Table 2. Much of the published available data regarding the pharmacokinetics of eszopiclone were based on preliminary research involving zopiclone.

Both zopiclone and eszopiclone are rapidly and extensively absorbed following oral administration. Following oral administration of a 7.5 mg zopiclone dose, peak plasma concentrations of 60 ng/ml are reached in less than 2 h [33]. Following a single 3 mg dose of eszopiclone, peak plasma concentrations are attained at approximately 1 h (T_{\max}) [34, 35]. The bioavailability of oral racemic zopiclone is ~80% [36]. Ingestion of eszopiclone with or shortly after a high fat meal delays absorption and increases T_{\max} by approximately 1 h, and reduces the C_{\max} by 21%, with no effect on the AUC_{0-24} , suggesting that the onset of action of eszopiclone may be delayed when administered with food [34].

The binding of racemic zopiclone to human plasma protein, albumin and alpha 1-acid glycoprotein, is stereoselective [37]. Zopiclone and eszopiclone weakly bind to plasma protein (52–59%), and the high free fraction is consistent with a large Vd, suggesting that disposition of the drugs should not be affected by drug interactions due to competitive plasma protein binding. The blood-to-plasma ratio is <1 for both eszopiclone and racemic zopiclone, indicating no selective uptake by red blood cells.

Zopiclone undergoes stereoconversion and is stereospecifically distributed to the brain [15]. Both drugs are widely distributed in body tissues, including the brain, with the volume of distribution (Vd) reported to be ~140 L and ~100 L for zopiclone

Table 2 Pharmacokinetic parameters of racemic zopiclone and its two enantiomers, S(+)-zopiclone and R(–)-zopiclone* [16, 28]

Parameter	Racemic zopiclone	S(+)-Zopiclone (eszopiclone)	R(–)-Zopiclone
$t_{1/2}$, eL (h)	5.6	6.5	3.8
$t_{1/2}$, abs (h)	0.58	0.61	0.47
Vd/F (l)	140.0	98.6	192.8
T_{\max} (h)	1.5	1.6	1.5
C_{\max} (ng/ml)	131	87	44
AUC (ng/ml ⁻¹ h)	865	691	210
Protein bound (%)	45	55	NA
Total Cl/F (L/h)	18.4	11.7	39.6
Renal Cl/F (L/h)	0.70	0.64	0.76

*After oral administration of a single 15-mg dose of racemic zopiclone (twice the usual therapeutic regimen) [16, 28]

$t_{1/2}$ *el* elimination of half-life; $t_{1/2}$ *abs* absorption half-life; Vd/F mean apparent volume of distribution; T_{\max} time to reach peak concentration; C_{\max} peak concentration; AUC area under the concentration curve; Total Cl/F total body clearance; Renal Cl/F renal clearance; protein bound (%) percentage bound to plasma protein; NA data not available

and eszopiclone, respectively [38]. Both drugs are eliminated in urine, saliva, and breast milk. Saliva concentrations of the drugs exceed those in plasma and may lead to a bitter taste [39]. Dosing studies in healthy adults have shown that both zopiclone (7.5 mg dose) and eszopiclone (1–6 mg) do not accumulate with once-daily dosing following repeated daily administration.

Both zopiclone and eszopiclone are extensively metabolized by oxidation and demethylation, and in vitro studies have shown CYP3A4 and CYP1E2 to play the most significant role in their metabolism. As racemic zopiclone is extensively metabolized in the liver to the active *N*-oxide-zopiclone and inactive *N*-desmethyl-zopiclone metabolites, it retains its chiral center; thus the two metabolites are themselves chiral and possess two enantiomers [40]. The sedative and anxiolytic activities of racemic zopiclone are produced mainly by the (S)-enantiomer. Eszopiclone is extensively metabolized and the two primary metabolites are (S)-zopiclone-*N*-oxide and (S)-*N*-desmethyl zopiclone. The former compound does not demonstrate significant binding to GABA receptors while the latter compound is the major metabolite, which binds to these receptors with substantially lower potency than the parent compound. Both eszopiclone and its metabolites have small Lowest Unoccupied Molecular Orbital (LUMO) and Highest Occupied Molecular Orbital (HOMO) energy differences, indicating that the compounds would be kinetically labile with (S)-zopiclone-*N*-oxide being most reactive. The molecular surfaces of eszopiclone and its two metabolites possess significant amounts of electron-deficient regions so that the compounds, especially (S)-zopiclone-*N*-oxide, may react readily with cellular nucleophiles such as glutathione and nucleobases in DNA. This would cause depletion of glutathione and oxidation of nucleobases, the former would induce cellular toxicity as a result of oxidative stress, and the latter could lead to DNA damage associated with oxidation of nucleobases [41].

Compared to R(–)-zopiclone, S(+)-zopiclone has a lower apparent V_d , a slower apparent oral clearance, and thus a longer terminal elimination half-life ($t_{1/2}$). Mean plasma-concentration-time curves for R(–)-zopiclone are reportedly lower vs. those for S(+)-zopiclone, resulting in lower values for maximum plasma concentration (C_{max}) and area under the concentration (AUC) [16]. Data from one pharmacokinetic study noted that the plasma concentration of (S)-zopiclone was approximately fourfold greater at 30 min following administration of eszopiclone compared with racemic zopiclone. A stereoselective metabolism is proposed as a possible explanation for the slower elimination of S(+)-zopiclone, since no differences in renal clearance have been noted for R(–)- and S(+)-zopiclone. Data from one pharmacodynamic study suggested that when eszopiclone plasma levels were ≤ 10 ng/ml, minimal hypnotic effects were observed on EEG and little cognitive impairment was observed. The point at which (S)-zopiclone plasma levels fall to 10 ng/ml is approximately 8 h for eszopiclone 3.5 mg compared to >9 h for 7.5 mg racemic zopiclone [24]. This suggests that patients who ingest zopiclone as compared to eszopiclone may be at greater risk for residual effects for at least 1 h longer in the morning following nighttime administration of the hypnotic.

Zopiclone elimination half-life ($t_{1/2}$) in healthy individuals has ranged from 3.5 to 6.5 h, and in elderly subjects, the $t_{1/2}$ increases to ~ 7 h [33]. The $t_{1/2}$ of

eszopiclone is approximately 6 h in adults [34, 35]. Pharmacokinetic studies of eszopiclone in elderly patients noted an increase of 41% in AUC without altering peak concentrations, and an increase in $t_{1/2}$ to 9 h compared with nonelderly adults [18]. Following oral administration, less than 10% of a zopiclone or eszopiclone dose is excreted in the urine as the parent drug. Gender and race have not been shown to interact with pharmacokinetics of zopiclone or eszopiclone.

In patients with hepatic insufficiency, the $t_{1/2}$ of zopiclone was substantially prolonged (~ 12 h) and T_{\max} was delayed (3.5 h), reflecting the reduction in hepatic metabolic clearance of racemic zopiclone in patients with cirrhosis as noted by the decrease in the metabolic ratio of the two main metabolites in patients with compromised hepatic function [36]. Although no dose adjustment with eszopiclone appears necessary for patients with mild or moderate hepatic impairment, patients with severe liver disease should not receive eszopiclone in doses greater than 2 mg as total systemic exposure was noted to increase by twofold in patients with severe hepatic impairment, compared to healthy volunteers.

In patients with mild to moderate renal insufficiency, the pharmacokinetics of zopiclone and eszopiclone were not affected. Studies in patients with severe renal impairment have shown inconsistent results possibly reflecting the large interindividual variability noted in the pharmacokinetics of zopiclone. Data from patients with severe chronic renal failure on zopiclone noted increased AUC and steady-state minimum plasma concentrations values than those observed in healthy volunteers, along with higher $t_{1/2}$ (~ 9 h); however, accumulation ratios did not differ between the two groups [42]. Other studies in patients with end-stage renal disease have noted only slight differences in the AUC and $t_{1/2}$ following zopiclone ingestion [43]. Hemodialysis does not appear to increase the plasma clearance of zopiclone. Since less than 10% of the orally administered eszopiclone dose is excreted unchanged in the urine as parent compound, no dosage adjustment appears necessary in patients with renal impairment. However, such patients need to be monitored [18].

5 Drug Interactions with Zopiclone and Eszopiclone

The pharmacological profile of racemic zopiclone is well documented, and the removal of the (R)-isomer did not unmask any new pharmacologic or toxicologic properties of eszopiclone; therefore, additional risks of pharmacodynamic interactions were not expected. Since both zopiclone and eszopiclone are metabolized by CYP3A4 and CYP2E1, caution is recommended when drugs that induce or inhibit these isozymes are coadministered. Potent CYP3A4 inhibitors (e.g., azole antifungals, amiodarone, cimetidine, fluoxamine, diltiazem, verapamil, erythromycin, clarithromycin, nefazodone, ritonavir, nelfinavir, and grapefruit juice) may increase the effect of zopiclone or eszopiclone if coadministered with the sedative hypnotics. Hence, when zopiclone or eszopiclone is used concomitantly with any of these drugs, a lower dose of the sedative hypnotic should be prescribed. Likewise,

CYP3A4 inducers (e.g., aminoglutethimide, carbamazepine, phenytoin, phenobarbital, rifabutin, rifampin, nevirapine, nafcillin, St. John's wort), if used concomitantly with zopiclone or eszopiclone, may decrease the effect of the sedative hypnotic, necessitating some patients to require a higher dose of either zopiclone or eszopiclone.

Zopiclone and eszopiclone's disposition should not be affected by drug interactions secondary to protein binding, as both drugs are weakly bound to plasma proteins.

Drugs that affect gastric emptying may alter the hypnotic efficacy of either zopiclone or eszopiclone. For example, intravenous metoclopramide increased plasma zopiclone levels while intravenous atropine reduced plasma zopiclone levels in healthy volunteers [44].

Although zopiclone and eszopiclone are chemically unrelated to benzodiazepines, barbiturates, or other drugs with known hypnotic properties, coadministration of these drugs may result in additive or enhanced sedative effects. Likewise, zopiclone and eszopiclone may produce additive CNS depressant effects when administered with other psychotropic agents, anticonvulsants, antihistamines, alcohol, and other agents known to produce CNS depression.

6 Effect on Sleep Stages

Like zaleplon, zolpidem [45], and ramelteon [46], zopiclone and eszopiclone essentially preserved natural sleep architecture, but did show an increase in stage 2 sleep during the clinical trials. Numerous studies in healthy volunteers or patients with insomnia have assessed the effects of zopiclone on sleep architecture. However, the results from these studies have been inconsistent, citing varying effects on aspects such as onset and duration of delta sleep, slow-wave sleep, and REM sleep. Clinical trials in sleep laboratories have noted that zopiclone leads to an increase in total sleep duration, a decrease of stage 1 sleep, and increase in stage 2, delta sleep, and slow-wave sleep. Additionally, some reports show that zopiclone reduces the total amount of time spent in REM sleep as well as delaying its onset [47–49]. Zopiclone has been noted to induce a highly significant reduction in increased values of cyclic alternating pattern (CAP) rate in response to white noise. CAP is a physiological electroencephalographic component that measures the amount of arousal instability during Non-REM sleep and subsequently, subjective assessment of sleep quality [50].

In clinical trials, the proportion of time spent in stage 1, delta sleep, slow-wave sleep, and REM sleep did not differ significantly and sleep architecture was essentially preserved following eszopiclone administration, being comparable to placebo for all treatment groups (1 mg, 2 mg, 3 mg) [51, 52], with the exception of eszopiclone 3.5 mg [53]. This higher dose group (3.5 mg) experienced a significant decrease in total time spent in REM, and in NREM stage 1 sleep, with a significant increase in total time spent in NREM stage 2. No significant differences were noted

in the restorative stages of sleep (delta sleep, slow-wave sleep) between all active treatment groups and the placebo group; however, significant increases in Stage 2 were noted with both the 2 mg and 3 mg dose of eszopiclone compared to placebo.

In animal studies involving aged guinea pigs, eszopiclone compared to zolpidem had a more rapid onset of hypnotic action and increased the amount spent in NREM sleep. A significant increase in the latency to REM sleep was also observed with eszopiclone but not with zolpidem. Additionally, eszopiclone produced an increase in EEG delta power and decrease in the theta band, whereas zolpidem had no effect on any of the frequency bands analyzed. The relative binding properties of eszopiclone and zolpidem and the reduction in the expression of specific subunits of GABA_A receptors in aged animals may account for the differences in the effects produced by eszopiclone and zolpidem [54].

7 Clinical Trials

7.1 Drug Development History

Zopiclone, already marketed in approximately 80 countries worldwide under the trade names of ImovaneTM and AmobanTM by Rhone-Poulenc Rorer (RPR), a unit of Phone-Poulenc SA, was not marketed in the United States but was originally claimed by RPR in US-03862149 in 1975, which expired in 1994 [55, 56]. Widely used in Europe since 1987 with over 20 million patient-years of experience, zopiclone is marketed as 7.5 mg tablets that contain 3.75 mg of S-zopiclone, more than the highest commercially available 3 mg dose of eszopiclone available in the United States. When the original developer of a pharmaceutical agent does not patent individual enantiomers, then third party companies, such as Sepracor, are able to enter into licensing agreements with the company that originally marketed the racemic compound and develop the single isomer or active metabolite versions of leading pharmaceutical products.

In October 1999, Phase I trials were underway following an Investigation New Drug (IND) filing in the second quarter of 1999. At that time, the manufacturer, Sepracor, entered into an agreement with Rhone-Poulenc Rorer SA for the exclusive license to the preclinical, clinical, and postmarketing surveillance data relating to zopiclone, its isomers, and metabolites. In December 1999, Phase II trials commenced, and in August 2000, the company had completed a large-scale efficacy study in transient insomnia, which noted that patients treated with eszopiclone successfully achieved all efficacy endpoints, including rapid onset of action and long duration of activity [55].

In January 2000, the manufacturer initiated a larger efficacy Phase II/III trial, which was completed by August of that year, involving 400 patients with insomnia. It was shown that the drug was well tolerated and had the potential to rapidly induce sleep, prevent nocturnal awakenings, and did not exhibit residual, next-day

hangover effects. By September 2000, patient enrollment for a second Phase III study was completed, and the trial initiation was planned for October 2000. Three hundred patients with insomnia demonstrated again that the drug had the potential to induce sleep with shorter duration of action, without residual, next-day hangover effects. Additionally, the sleep induced by eszopiclone was rapid and its optimal duration of action prevented nocturnal awakenings. Four Phase IIIB/IV trials were initiated in December 2003 to evaluate the efficacy of eszopiclone in the treatment of insomnia in patients with chronic insomnia, depression, rheumatoid arthritis, and in women with symptoms of perimenopause.

On December 15, 2004, the U.S. Food and Drug Administration (FDA) approved the New Drug Application (NDA) for LunestaTM, brand eszopiclone, formerly referred to as EstorraTM for the management of insomnia in adult patients ≥ 18 years of age. The NDA contained data from a total of 24 clinical trials and more than 60 preclinical studies. In total, $>2,700$ patients participated in the clinical development program for eszopiclone (Phases I through III), where approximately 1,550 patients were exposed to eszopiclone in placebo-controlled clinical efficacy trials involving approximately 2,120 patients.

These 24 clinical trials evaluated the efficacy and safety of eszopiclone for the treatment of insomnia in adult (aged 21–64 years) and elderly (aged 65–86 years) patients. Sixteen Phase I trials involving 593 subjects were conducted to gather information on drug interaction and special population studies. Two Phase II trials involving 25 subjects were undertaken to assess next-day residual effects in healthy subjects and in patients with chronic insomnia. Six Phase III trials included the following: one trial in transient insomnia was conducted in 436 healthy subjects using the first-night effect model, three trials in 1,164 adults with chronic, primary insomnia, and two trials in 526 elderly patients with chronic, primary insomnia.

In 2005, eszopiclone was the first sedative-hypnotic to receive approved labeling from the FDA for long-term use in chronic insomnia, with no proposed restrictions as to how long it may be utilized. Since then, both zolpidem CR and ramelteon no longer have any implied limitation on their duration of use and have this open-ended indication.

In July 2007, Sepracor Inc. submitted a marketing authorization application (MAA) for LuniviaTM brand eszopiclone to the European Medicines Agency (EMA) for review under the centralized process. The MAA is the EU equivalent of a NDA in the United States. This procedure allows for a single, coordinated technical review conducted by the EMA on behalf of all European Union (EU) member states. Experts from national authorities act as lead reviewers and approval of the MAA would allow authorization to market LuniviaTM in as many as 27 EU member countries. Included in the MAA were results from 122 preclinical and 35 clinical trials, which included more than 5,500 adult and older adult (>65 years of age) subjects, including patients with transient and chronic insomnia. Additionally included as part of the submission were studies of LunestaTM in patients with insomnia and coexisting conditions, two 6-month, placebo-controlled studies in primary insomnia, as well as two driving studies showing no effect on next-day driving following eszopiclone use in healthy subjects or patients with insomnia.

Also, in July 2007, Eisai Co., Ltd. and Sepracor Inc. signed an in-licensing agreement for eszopiclone, allowing Eisai to assume exclusive rights for development and marketing eszopiclone as LuniviaTM in Japan. At the same time, a Phase I clinical study with eszopiclone was ongoing in Japan. Later that year, Sepracor Inc. and GlaxoSmithKline (GSK) announced an agreement for commercialization LuniviaTM for all markets worldwide, excluding the United States, Canada, Mexico, and Japan.

On October 23, 2008, the EMEA's Committee for Medicinal Products for Human Use (CHMP) recommended that a marketing authorization be granted for LuniviaTM 2 mg and 3 mg, "intended for the treatment of insomnia, including difficulty falling asleep, nocturnal awakening or early awakening, in adults" [57]. However, the CHMP determined that no meaningful clinical difference could be established between eszopiclone and zopiclone regarding safety and efficacy, and recommended that the medicine should not be regarded as containing a new active substance, and as such should not be granted "new active substance" status. This prevented LuniviaTM from benefiting from 10 years of "market exclusivity," a time period during which other companies would be prevented from marketing their generic versions of eszopiclone. In February 2009, the CHMP reconfirmed its earlier opinion upon reexamination procedure, and in April 2009, Sepracor withdrew its application to LuniviaTM in Europe, noting that the commercial viability of launching the product in the EU was compromised (EMA 2009) [58].

Sepracor is currently developing SEP 0227018, a new formulation of the zopiclone isomer, eszopiclone, for the potential treatment of insomnia. It is being studied for potentially improved efficacy, and currently, the compound is undergoing Phase II clinical development and evaluation in the United States. In March 2009, Sepracor filed a lawsuit in the US District Court for the District of New Jersey against those companies who have filed abbreviated new drug applications (ANDAs) with Paragraph IV certificates for generic copies of eszopiclone. During the period of activities at this time, ANDA approval will be stayed until approximately June 15, 2012 (or potentially 6 months thereafter if the manufacturer successfully acquires a pediatric exclusivity extension), or until a court determines that Sepracor's patents are invalid, unenforceable, or not infringed, whichever is earlier [59].

8 Clinical Efficacy

8.1 Noncomparative Studies and Comparisons with Placebo

Several noncomparative and placebo-controlled trials have demonstrated the hypnotic efficacy of zopiclone and eszopiclone in patients with insomnia [51–53, 60–67]. Since a very high placebo response is common in patients with insomnia, comparisons between placebo and zopiclone or eszopiclone have confirmed these

findings as the hypnotics produced significant improvements over placebo on measures of sleep latency and sleep maintenance.

8.2 *Zopiclone Data*

Studies comparing zopiclone 7.5 mg with placebo for 7–21 consecutive nights in patients with chronic insomnia noted that the hypnotic reduced sleep latency onset and number of awakenings (NAW), and increased total sleep time (TST) and sleep quality in more than 80% of patients [39], although most positive results were derived primarily from subjective assessments. Zopiclone's efficacy as a hypnotic over the dose range of 3.75–10 mg was evaluated in geriatric patients (mean age 81 years old) for 2 weeks. All patients on zopiclone slept better compared to placebo with only slight differences between the different dose levels with regards to sleep quantity and quality [61]. Data from 20,513 insomniac outpatients in a postmarketing study noted that efficacy of treatment as assessed by the physician and patient, respectively, after 21 days of treatment was rated as “good to excellent” in more than 80% and 83% of cases, and “poor or bad” in 4% and 5% of the cases [68].

8.3 *Eszopiclone Data*

One study evaluated the efficacy and safety of eszopiclone in healthy adults with normal sleep patterns and no history of insomnia or other significant illness, in a first-night effect model of transient insomnia [53]. Patients were randomized to eszopiclone 1 mg, 2 mg, 3 mg, or 3.5 mg, or placebo. The primary efficacy end-point, latency to persistent sleep as assessed via polysomnography (PSG), was significantly shorter in patients who received eszopiclone 2 mg, 3 mg, and 3.5 mg compared to patients who received placebo. Wake after sleep onset (WASO) was significantly reduced in all patients taking eszopiclone compared with those taking placebo. All patients receiving eszopiclone reported fewer NAW, but the difference was significant for eszopiclone 3 mg and 3.5 mg compared to placebo. Sleep efficiency was improved with all doses of eszopiclone compared to placebo, and subjects' self-reported efficacy measures were consistent with PSG results.

Four studies have evaluated the safety and efficacy of eszopiclone in nonelderly patients with primary, chronic insomnia [51, 64–66]. Patients in all these studies met DSM-IV criteria for primary insomnia, had ≤ 6.5 h of sleep per night, had a sleep onset latency of ≥ 30 min, and had no other concurrent psychiatric disorders. The efficacy and tolerability of 44 consecutive days of eszopiclone 2 mg or 3 mg was assessed in one trial with PSG and subjective patient reports (next-morning questionnaires) [51]. Eszopiclone 2 mg and 3 mg, compared to placebo, significantly reduced the median double-blind average latency to persistent sleep (15.0 min, 13.1 min, and 29.0 min, respectively); hence patients receiving eszopiclone fell

asleep approximately 50% faster than those receiving placebo. Both doses of eszopiclone significantly improved the double-blind average sleep efficiency compared to placebo. Additionally, 68% of patients in the eszopiclone 3 mg group and 53% of patients in the eszopiclone 2 mg group had PSG-defined TST ≥ 7 h (the double-blind average), relative to 37% of patients in the placebo group. The double-blind average WASO for eszopiclone 3 mg was significantly reduced compared to placebo, but not with the 2 mg dose. No significant differences in double-blind mean PSG-defined NAW were noted between treatment and placebo groups. Results for the subjective nightly patient-reported sleep endpoints were consistent with the objective PSG findings.

Krystal and coworkers [64] assessed the long-term safety and efficacy of eszopiclone 3 mg in 788 nonelderly patients with primary insomnia in a 6-month outpatient trial carried out at 70 sites. By the first week, median time to sleep onset was 30 min for eszopiclone compared to 60 min in the placebo group. Eszopiclone maintained the half-hour sleep onset time throughout the trial; however, the difference compared to placebo decreased over the 6 month period. At the first week and each month of the study duration, eszopiclone compared to placebo led to significant and sustained improvements in sleep latency, WASO, TST, NAW, number of nights awakened per week, and quality of sleep. Although a limitation to this study is the lack of objective assessment via PSG to correlate with the subjective assessments reported by patients, this was the first study that empirically assessed the efficacy and safety of long-term treatment of chronic insomnia and the primary trial on which the FDA based the approval of eszopiclone for long-term use.

In a 6 month, open-label extension phase of the study described above, 471 patients (360 and 111 patients who were originally assigned to receive eszopiclone or placebo, respectively) were given eszopiclone 3 mg [65]. Sleep variables were assessed at weekly intervals via interactive voice response system (IVRS), and data from this trial provided further assessment of the efficacy and safety of eszopiclone in adults with primary insomnia for a total of 12 months of continuous nightly treatment. Patients previously treated with placebo reported significant decreases in sleep latency, WASO, NAW, and number of nights with awakenings at all monthly time points compared with their 6 month baseline. Additionally, increases in TST, and sleep quality, as well as improved ratings in daytime alertness, ability to function, and physical sense of well-being were noted at all monthly time points compared with open-label baseline. Significant improvements in sleep and daytime function were also sustained during the 6 months of open-label treatment for those who received eszopiclone during the double-blind trial. These patients reported significant decreases in sleep latency (months 8, 9, 10, and 12), WASO (months 8–12), NAW (months 9–12), and the number of nights with awakenings (months 8 and 10), and increases in TST and sleep quality at all time points.

Another long-term trial evaluated 6 months of eszopiclone 3 mg nightly treatment and noted significant improvement over placebo in sleep latency, and secondary parameters related to sleep maintenance, including WASO and TST [66]. Median sleep quality scores at 6 months were increased 59% from baseline in

patients treated with eszopiclone 3 mg compared with 31% in patients who received placebo. At all monthly assessment points, eszopiclone 3 mg produced consistent and sustained improvements across all sleep and daytime function parameters compared to placebo, except for NAW. At month 6, Insomnia Severity Index (ISI) scores were reduced to below clinically meaningful levels ($ISI \leq 7$) for 50% of patients treated with eszopiclone, compared to 19% of patients treated with placebo. For the month 1–6 average, the Medical Outcomes Study Short-Form Health Survey (SF-36) domains of physical functioning, vitality, and social functioning were improved in patients who received eszopiclone compared to placebo. Likewise, improvements were reported for all domains of the work limitations questionnaire with eszopiclone compared to placebo-treated patients for the double-blind month 1–6 average, and significant improvements with eszopiclone, relative to placebo, were observed at months 1, 3, and 6 for work productivity loss domain, months 1 and 6 for time demand, months 1 and 3 for physical demands, and month 3 for the output domain. This was the first study to demonstrate that long-term treatment of primary insomnia with eszopiclone 3 mg enhanced quality of life, reduced global insomnia severity, reduced work limitations, and improved quantitative, patient-reported sleep variables. Since the diagnosis of insomnia requires an associated daytime effect, improvements in quality of life and other functional outcomes are becoming more valuable in assessing efficacy of sedative-hypnotic agents [69].

Two separate 2-week trials of eszopiclone were conducted in chronic insomnia elderly patients [52, 67]. Both trials noted all doses of eszopiclone superior to placebo on measures of sleep latency, with the 2 mg dose superior to placebo on measures of sleep maintenance. The earlier study in elderly patients with chronic insomnia assessed the effect of eszopiclone 1 mg or 2 mg compared to placebo nightly for 2 weeks via patient-reported measures [67]. Eszopiclone 2 mg significantly improved sleep latency, WASO, TST, sleep quality, and depth of sleep compared with placebo over the 2 week period. Improvements in patient-reported measures with eszopiclone 2 mg were noted in several measures of daytime function, including improvements in daytime alertness and the sense of physical well-being. The NAW did not differ between eszopiclone 1 mg, 2 mg, and placebo groups. The average number of naps taken was significantly lower in the eszopiclone 2 mg group, and both doses of eszopiclone significantly reduced the cumulative duration of naps compared to placebo. The efficacy of eszopiclone 1 mg compared to placebo was limited to significantly decreasing sleep latency; otherwise, no significant impact on any sleep maintenance parameters was noted with this dose. The 2 mg dose appeared more effective than the 1 mg dose in elderly subjects, and it is believed that nighttime administration of eszopiclone 3 mg for adults and 2 mg for elderly patients will provide effective hypnotic activity.

The second study in elderly patients evaluated eszopiclone 2 mg in elderly patients in 49 sleep laboratories utilizing PSG and patient-reported assessments [52]. PSG change from baseline over the 2-week period was significantly improved over placebo in latency to persistent sleep (LPS), WASO, wake time during sleep, TST, and sleep efficiency. Patient-reported change from baseline over the

double-blind period noted significant improvement over placebo in sleep latency, WASO, and TST. Although PSG and patient-reported awakenings were reduced, statistical significance was not reached. Patients in the eszopiclone group noted a significant improvement in the mean change from baseline for the ISI total severity score, quality of sleep score (1.1 for eszopiclone, 0.6 for placebo), and the SF-36 physical functioning score. No significant differences were noted in the SF-36 vitality score between both groups. Although patients were discouraged from napping, among patients who did nap, eszopiclone was associated with a decrease in the cumulative number of naps (median: eszopiclone 2, placebo 3). No significant differences were observed in the duration of naps or morning sleepiness between eszopiclone and placebo.

In a recent longer-term, double-blind, randomized, safety and efficacy trial with elderly patients, eszopiclone 2 mg demonstrated significant improvements in measures of daytime function compared to placebo over a 12-week treatment period [70]. In this study, patients with stable medical abnormalities or chronic conditions were not excluded, and concomitant medications were used by 96% of patients overall, with lipid-modifying agents being the most common concomitant medications. These improvements were noted during the first 3 weeks of treatment and were sustained through the last 3 weeks of the double-blind period. Although both the number of naps and total daily nap time decreased from baseline for patients who received eszopiclone and placebo, these decreases were statistically significantly different between the two treatment groups during the first 3 weeks of the study. At week 12, patients administered eszopiclone reported statistically significant differences in measures of improved sleep quality, improvement in feeling refreshed/rested, reduced daytime fatigue, and reduced number of nights per week with sleep difficulties scores. Attention/concentration were also statistically significantly improved for eszopiclone-treated patients compared to placebo-treated patients, although no significant differences from placebo in ISI scores measuring attention, concentration, relationship enjoyment, or mood disturbance were observed. The SF-36 noted that patients who received eszopiclone, compared to those who received placebo, reported significantly improved general health and vitality at week 12. Patients treated with eszopiclone also showed general improvements from baseline in the social life and family life/home responsibilities items of the SDS versus patients treated with placebo.

Upon conclusion of this trial, patients received single-blind placebo for 2 weeks to assess rebound and withdrawal effects and then were followed for an additional 2 week evaluation period, in which patients received neither eszopiclone nor placebo, to assess the durability of the response [71]. Sleep latency, WASO, and TST were all statistically significantly improved compared to baseline measures following discontinuation of treatment, indicating absence of rebound insomnia, and no withdrawal symptoms were noted following discontinuation of treatment. The study results noted no statistically significant difference in benzodiazepine withdrawal symptoms questionnaire (BWSQ) (an assessment of symptoms associated with benzodiazepine discontinuation) scores for eszopiclone-treated patients at the end of week 12 relative to the end of the single-blind placebo administration

period at week 14. The BWSQ scores at week 14 were comparable for patients who received eszopiclone during the initial 12-week period and who received placebo throughout the study duration. The percentages of eszopiclone-treated patients with “no insomnia” and “sub-threshold insomnia,” as assessed via the ISI, declined from the end of the double-blind period to the end of the 4-week follow-up period; however, the percentages of patients in these categories were still higher at the end of the 4-week follow-up period than they were at baseline at the start of the study.

In a study evaluating next-day clinical residual effects of a single dose of eszopiclone 3 mg, participants in both Study 1 (healthy volunteers) and Study 2 (patients with primary insomnia) who received the hypnotic compared to placebo noted significant improvement in “ease of getting to sleep” and “quality of sleep” on subjective measures of sleep. Study 1 participants noted statistically significant increase in feelings of next-day sedation, relative to placebo; however, no such differences were noted in patients with primary insomnia. No differences were observed in coordination or mood between both treatment groups in both Study 1 and Study 2. Objective PSG measures in Study 2 patients with insomnia noted significant improvement in sleep efficiency, TST, sleep onset latency, WASO, and latency to persistent sleep for patients on eszopiclone compared to placebo [72].

8.4 *Comorbid Insomnia*

Comorbid insomnia is most often associated with medical and psychiatric disorders, such as neurologic disorders (e.g., stroke, dementia, restless leg syndrome, seizure disorder, Alzheimer disease, Parkinson disease), psychiatric (e.g., depression, anxiety, depression, psychosis, delirium), cardiovascular (e.g., heart failure, nocturnal angina), gastrointestinal disorders (e.g., gastroesophageal reflux disease, peptic ulcer disease), urinary (nocturia, incontinence, incomplete bladder emptying), pulmonary (e.g., nocturnal asthma, chronic obstructive pulmonary disease, allergic rhinitis), and disorders causing pain (e.g., rheumatoid arthritis, fibromyalgia). Approximately 50% of patients with other medical conditions experience insomnia, and emerging evidence demonstrates that for patients suffering from insomnia with other comorbid medical conditions, treating the insomnia and subsequent improvement in sleep may benefit the comorbid condition itself and improve the patient’s overall outcome [73].

Data from trials in insomnia as comorbid condition are also supportive of the efficacy of eszopiclone. There were several trials that enrolled patients with insomnia and comorbid conditions including major depressive disorder (MDD), generalized anxiety disorder (GAD), menopausal transition, rheumatoid arthritis, and obstructive sleep apnea syndrome (OSAS). The manufacturer has commenced enrollment of its Pan-European 6-month study of eszopiclone coadministered with venlafaxine XR in patients with insomnia and coexisting MDD. This 640 patient trial will assess the potential benefit of eszopiclone in reducing symptoms of

depression and in preventing relapse. Additional ongoing clinical trials are under-way evaluating the use of eszopiclone in the treatment of insomnia in patients with coexisting disorders such as Parkinson's Disease, fibromyalgia, and low back pain.

8.5 *Effects on Depression*

Recent studies suggest an association between major depressive disorder (MDD) and the α_3 subunit of the GABA receptor; and the greater α_3 subunit effects of eszopiclone are consistent with the differential effects of eszopiclone and racemic zopiclone on antidepressant outcomes. Data from clinical trials indicate that eszopiclone coadministered with antidepressants has greater effects on depression outcomes than either racemic zopiclone or zolpidem, which have relatively less α_3 activity.

The efficacy of adding eszopiclone to fluoxetine in patients who met the DSM-IV criteria for both major depressive disorder (MDD) and insomnia was assessed in a multisite study [74]. Patients received morning fluoxetine (dose range: 20–40 mg/day) and were randomized to nightly eszopiclone 3 mg or placebo for 8 weeks. Patient-reported assessments of various sleep parameters and daytime functioning were obtained with IVRS, while depressive symptoms were assessed with the 17-item Hamilton Rating Scale for Depression (HAM-D-17), the 6-item Bech and Maier subscales of the HAM-D-17 (HAM-D-6), and Clinical Global Impression Improvement (CGI-I) and Severity (CGI-S) scales (The HAM-D-17 is the standard scale utilized in research studies to assess depression; a lower score indicates fewer depressive symptoms. The CGI-I and CGI-S scales are used by clinicians to assess improvement in a patient's MDD symptoms, and the severity of their depression at various time points, respectively).

Beginning on the first treatment night and continuing throughout the double-blind treatment period, patients treated with eszopiclone, compared to placebo, had significantly greater improvements on all sleep efficacy endpoints: sleep onset, WASO, TST. Additionally, these patients reported improved sleep quality and depth of sleep at all time points. Measures of next-day functioning were significantly improved with eszopiclone compared to placebo. ISI total severity scores across the double-blind period for patients treated with eszopiclone were noted to have significantly greater reductions at all time points, and at week 8, 50% of patients in the eszopiclone group compared to 33% in the placebo group had no clinically meaningful insomnia (ISI total score ≤ 7).

Patients coadministered eszopiclone with fluoxetine demonstrated statistically significant improvements in clinician-rated depression as noted by reduced HAM-D-17 scores at week 4 and a progressive improvement at week 8. After removing three insomnia-related items from the HAM-D-17, reduction in HAM-D-17 scores remained significant for patients treated with eszopiclone at week 8, but not at week 4. By the end of the double-blind period, patients who were coadministered eszopiclone had significantly more responders (patients with $\geq 50\%$ improvement in HAM-D-17 score, 59% vs. 48%, respectively), and remitters (patients with

HAM-D-17 ≤ 7 ; 42% vs. 33%, respectively) compared to those coadministered placebo. Additionally, patients treated with eszopiclone had significantly shorter times to antidepressant response based on the CGI-I and CGI-S scores. No significant differences were noted between treatment groups at any assessment point in patient-reported Bech and Maier subscales of the HAM-D-17. Throughout the double-blind period, eszopiclone coadministered with fluoxetine was well tolerated and discontinuing eszopiclone did not adversely affect the antidepressant response.

Patients who discontinued from eszopiclone maintained improvements from baseline in sleep onset, WASO, and TST during the 2 week placebo runout period. Patient-reported daytime functioning was maintained in patients who received eszopiclone and fluoxetine following the discontinuation of the sedative–hypnotic, and observed scores at week 10 were similar to week 8 for daytime alertness, concentration, physical well-being, and ability to function. Physician-assessed CGI-I scores in sleep and depressive symptoms were maintained. Significantly lower HAM-D-17 scores were observed in the cotherapy group with the sedative–hypnotic compared with the monotherapy group at week 8 and were maintained at week 10 [75].

8.6 *Effects on Anxiety*

Results of a study in subjects with GAD [76] noted that administration of zopiclone 7.5 mg as monotherapy at night did not demonstrate any improvement in the HAM-A total score compared to placebo at week 4. Two previous double-blind crossover studies support the findings of a lack of anxiolytic effect associated with racemic zopiclone [77, 78]. Evaluation of theta activity utilizing EEG as an indicator of anxiolytic effects in healthy male subjects administered placebo, diazepam 5 mg, zopiclone 5 mg, and zopiclone 10 mg was assessed. No effect was observed with zopiclone 5 mg (equivalent to 2.5 mg of the (S)-isomer) on EEG theta activity or as measured by the State Trait Anxiety Inventory (STAI) scale. Zopiclone 10 mg only slightly modified the frontal midline theta activity, while diazepam increased markedly the frontal midline theta activity, suggesting that anxiolytic potential of zopiclone in humans is probably low.

Sepracor evaluated daytime administration of eszopiclone 0.3 mg, 0.6 mg, 0.9 mg, or 2.0 mg or placebo in healthy male subjects utilizing quantitative electroencephalography (qEEG), auditory P300 (evoked potential), and cognitive functioning from 0.5 to 12 h following daytime dosing [24]. Evaluation of EEG frequency bands was used to distinguish anxiolytic activity, based on analysis of beta and theta EEG activity, from sedative activity, based on analysis of alpha and delta EEG activity. All doses of eszopiclone based on qEEG analysis significantly increased beta EEG activity compared with placebo in a dose-dependent manner, demonstrating an anxiolytic effect within 0.5 h postdose. Also noted was a dose-dependent decrease in theta power, which also indicates an anxiolytic effect. Doses below 2 mg did not exhibit significant effects on arousal or sedation; however, the

2 mg dose was noted to increase in delta power and decrease in alpha power indicating significant impairment of the subject's arousal level.

Eszopiclone coadministered with escitalopram in treating patients with insomnia comorbid with Generalized Anxiety Disorder (GAD) was evaluated in a multicenter study [79]. The primary endpoint noted that treatment with eszopiclone/escitalopram resulted in significantly reduced sleep latency averaged across the double-blind period compared to treatment with placebo/escitalopram (−25 min vs. −11 min, respectively). Additionally, significant improvements in secondary key endpoints were noted in the eszopiclone/escitalopram treatment group compared to the monotherapy-treated group, respectively: TST averaged across the double-blind period (+61 min vs. +35 min), change from baseline Hamilton Anxiety Rating Scale (HAM-A) at week 8 (−12 vs. −11), and 50% reduction from baseline in HAM-A at week 8 (63% vs. 49%). Other secondary endpoints that did not yield significant results included time to onset of anxiolytic response based on CGI-I score ≤ 2 (18 days vs. 28 days) and change from baseline CGI-S at week 8 (−1.7 vs. −1.6).

Total HAM-D-17 scores (including and excluding insomnia items) significantly improved from baseline at weeks 4, 6, and 8 in the eszopiclone/escitalopram group relative to the placebo/escitalopram group. However, at week 10, the total HAM-D-17 scores, including and excluding sleep items, were not significantly different between the two treatment groups. At week 8, patients treated with eszopiclone/escitalopram had higher HAM-A response compared to patients treated with placebo/escitalopram (63% vs. 49%, respectively), although rates of remission did not differ significantly between the two treatment groups (42% vs. 36%, respectively). Improvements in total HAM-A scores for each week of the study were noted in patients who were administered eszopiclone/escitalopram cotherapy, compared to the escitalopram monotherapy group, and at weeks 4 through 10, when the insomnia item was excluded. At week 8, significantly more patients in the eszopiclone/escitalopram group had no clinically meaningful insomnia based on an ISI score ≤ 7 compared to patients in the placebo/escitalopram group (47% vs. 33%, respectively). At every point in the double-blind period, CGI-I scores were improved with eszopiclone/escitalopram, although CGI-S scores did not significantly differ between treatment groups after week 1. Administration of eszopiclone with escitalopram also resulted in significant patient-reported improvements from baseline in daytime symptoms of insomnia, including daytime alertness, ability to function, ability to concentrate, and physical well-being compared to placebo administered with escitalopram, when averaged over the treatment period.

Sepracor has completed the analysis and validation of the preliminary results of a Phase II, 440 patient trial evaluating the efficacy and safety of SEP-225441 (a modified release formulation of eszopiclone) for the treatment of GAD. The study did not meet its primary endpoint, which was a reduction in symptoms of GAD, as assessed via the HAM-A. Currently, the manufacturer is further analyzing the data to determine whether SEP-225441 warrants further clinical development [80].

Data suggest that exposure to low levels of eszopiclone during the elimination phase, the next day following nighttime administration, may have an anxiolytic effect that contributed to the improvements noted on measures of anxiety in clinical

trials. However, much higher doses of racemic zopiclone may be necessary to produce an anxiolytic effect and this effect is not distinct from its sedative effects, possibly related to the interference of the (R)- isomer with respect to the pharmacological activity of the (S)-isomer at the α_2 and α_3 receptor subunits.

8.7 Effects on Perimenopausal and Menopausal Women

A multicenter study evaluated the safety and efficacy of eszopiclone for the treatment of insomnia in perimenopausal and early menopausal women confirmed on the Greene Climacteric Scale (GCS) suffering from insomnia [81]. Eligible patients reported sleep latency ≥ 45 min and sleep duration ≤ 6 h, for greater than three times per week for 1 month. Insomnia symptoms postdated onset of perimenopausal symptoms, with no other cause of secondary insomnia.

At baseline, the majority of patients had an SL of 45–59 min, sleep duration of 5–6 h per night, three to five nocturnal awakenings per night, and WASO of 30–60 min per night. Eszopiclone significantly decreased SL and WASO relative to placebo at all weekly timepoints. Over the 4-week study period, patients treated with eszopiclone compared to placebo, respectively, had a mean reduction in SL (25.8 min vs. 10.1 min), mean reduction in WASO (30.9 min vs. 16 min), and mean increase in TST (56.6 min vs. 33.6 min). Additionally, compared to placebo, patients who received eszopiclone also noted improved sleep quality, depth of sleep, daytime alertness, ability to function, ability to concentrate, and physical well-being.

At week 4, study participants treated with eszopiclone reported greater improvement in several menopause-related measures including fewer awakenings, and fewer awakenings due to hot flushes, compared to placebo treated patients. More eszopiclone-treated patients reported no clinically significant insomnia (ISI total score ≤ 7) compared to patients who received placebo (58% vs. 35%, respectively). After receiving eszopiclone for 4 weeks, patients noted improvements in vasomotor and physical subscores of the menopause-specific quality of life (MenQOL) questionnaire; and lower Greene Climacteric Scale (GCS) total score, including improvements in vasomotor and psychological domains. Additionally, at week 4, patients treated with eszopiclone, relative to placebo, showed significant improvement in mood, as assessed by the Montgomery Asberg Depression Rating Scale (MADRS), and had significant improvement in the family life/home domains of the Sheehan Disability Scale (SDS). In addition to patient-reported benefits, changes in the Physician Global Assessment of menopausal symptoms at week 4 noted more patients treated with eszopiclone were “very much improved” or “much improved,” and fewer patients had “no change” in menopause symptoms relative to placebo patients. However, no significant differences were noted between treatments with regard to changes from baseline in menopause symptoms following a single-blind placebo runout period for 1 week after the 4-week treatment period.

Although zopiclone has not been evaluated in any placebo-controlled study in depressed patients, a double-blind placebo and active controlled 2-week crossover study of racemic zopiclone 7.5 mg in menopausal women (40–60 years of age) demonstrated no effect on the Profile of Mood Scale (PMOS) [24]. Eszopiclone, however, has demonstrated improvements in women with underlying depressive symptoms in the perimenopausal transition as measured by the change from baseline MADRS score.

8.8 Effects on Pain (*Rheumatoid Arthritis, Fibromyalgia*)

Differences noted in zopiclone and eszopiclone's effects in subjects with chronic pain may be due to differences in GABA pharmacology related to the α_2 and α_3 subunits. Analgesic effects are associated with agents that are selective for GABA α_2 and α_3 subtypes, which may be related to the diminished pain signals to the brain as well as reduction in the associated emotional or anxiogenic aspects of pain.

Several studies have evaluated zopiclone's effects in patients with insomnia and comorbid chronic painful conditions including rheumatoid arthritis [82, 83] and fibromyalgia [84]. No improvements in measures of general pain, morning stiffness, tenderpoint sensitivity, or other pain measures following 2–8 weeks of treatment with zopiclone 7.5 mg relative to placebo were observed in all three studies. However, one study in fibromyalgia patients noted that although improvements in sleep were observed in patients who were administered zopiclone, the comorbid pain worsened, as most patients who received zopiclone had worsened tenderpoint sensitivity and global pain drawing scores at 8 weeks compared with placebo, most placebo-treated patients demonstrating improvements in these pain scores [83]. A Phase IV study is underway, assessing eszopiclone for the treatment of insomnia and other symptoms of fibromyalgia. The primary outcome to be measured is TST as recorded in patient diaries, with secondary outcomes including assessment of WASO, sleep quality, clinician-rated overall severity of fibromyalgia, and Fibromyalgia Impact Questionnaire.

A multicenter trial evaluated the effects of eszopiclone on sleep measures, rheumatoid arthritis (RA), and Quality of Life (QoL) assessments, and evaluated the safety of the eszopiclone in patients with RA and coexisting insomnia [85]. Patients diagnosed with RA were administered nightly eszopiclone 3 mg for 4 weeks, followed by a 2-week single-blind placebo runout period. Subjective pain severity was assessed via IVRS. Assessments of RA symptoms included the Arthritis Self-Efficiency Scale (ASES) and the American College of Rheumatology (ACR) response criteria.

Eszopiclone-treated patients reported significant improvement in their SL, WASO, NAW, TST, and sleep depth and quality compared to placebo. Additionally, significant improvements were noted in next-day performance measures as indicated by ratings of daytime alertness, ability to concentrate, and ability to function. At week 4, the ISI total scores for patients treated with eszopiclone

compared to placebo were significantly better, as were individual items of sleep quality, feeling refreshed/rested, daytime fatigue, relationship enjoyment, and sleep difficulties. Additionally, at week 4, a significantly greater number of patients treated with eszopiclone had no insomnia (ISI total score ≤ 7), compared with patients who received placebo (47.9% vs. 30.4%).

Mean change from baseline scores on the ASES at week 4 for patients receiving eszopiclone were clinically and statistically significant for overall score, pain, and pain in association with symptoms compared to placebo. Mean change from baseline IVRS assessments over the double-blind treatment period in patients treated with eszopiclone compared to placebo noted improvement in pain severity from the previous day and in ability to function. Although no significant differences were noted in the duration or severity of morning stiffness, patients treated with eszopiclone reported significant reduction in pain severity and the number of tender joints. At week 4, eszopiclone relative to placebo significantly improved the mean change from baseline role-physical and bodily pain parameters of the SF-36 Acute Health Survey.

8.9 Use in Obstructive Sleep Apnea and Pulmonary Disease

An undesired adverse event of some sedative–hypnotic agents is respiratory depression. A review of various hypnotics used in chronic obstructive pulmonary disease (COPD) patients [86] noted that zopiclone 7.5–10 mg was found to have no significant effect on ventilatory drive and central control of breathing in normal subjects or in patients with mild to moderate COPD, although one small study did note a nonsignificant trend towards an increased number and duration of apneic episodes in patients taking zopiclone (5–10 mg).

Eszopiclone administered in 14 healthy male volunteers (aged 20–43 years) at doses up to 7 mg orally did not produce respiratory depressant effects when compared to codeine 60 mg and placebo [87]. Ventilatory response and mouth occlusion pressure to carbon dioxide (CO₂) were assessed at predose and at 2 h, 4 h, and 6 h after dose administration. Codeine 60 mg produced significantly reduced ventilatory response to CO₂ 2 h after dose administration ($p < 0.05$) but not after 4 h and 6 h. Eszopiclone at doses of 3 mg and 7 mg (more than twice the potency of the highest commercially available dose) did not affect ventilatory response to CO₂ and did not produce respiratory depression. Nonetheless, caution should be used when administering eszopiclone to patients with compromised respiratory function.

A crossover study evaluated the effects of eszopiclone on measures of respiration and sleep using PSG readings in patients with mild-to-moderate obstructive sleep apnea syndrome (OSAS) [88]. For two consecutive nights, 21 adults (males – 68%) with an Apnea-Hypopnea Index (AHI) ≥ 10 and ≤ 40 , who used continuous positive airway pressure (CPAP) nightly, randomly received eszopiclone 3 mg or placebo with a 3–5 day washout between visits. The presence or absence of insomnia was not an entry requirement and CPAP was not allowed during the

2 nights of PSG recording. The primary endpoint, mean total AHI, did not differ significantly between patients who received eszopiclone or placebo (16.7 vs. 16.5). Likewise, no significant differences between both treatment groups were noted in total arousals, respiratory arousals, duration of apnea and hypopnea episodes, or oxygen saturation. Spontaneous arousals were significantly reduced with eszopiclone relative to placebo (11.4 vs. 13.6, respectively). Additionally, PSG noted improvements in sleep efficiency, WASO, and wake time during sleep following eszopiclone treatment. There was no statistically significant difference between eszopiclone and placebo in objective LPS or in the number of awakenings at any time point during the study. Since sleep deprivation has been linked to weight gain, which may exacerbate sleep apnea, further studies are warranted to study eszopiclone as adjunct therapy in patients with mild to moderate OSAS.

A prospective, double-blind, placebo-controlled study in an academic multidisciplinary sleep center setting demonstrated that eszopiclone 3 mg did not worsen the severity of sleep-disordered breathing. Pretreatment with eszopiclone 3 mg improved a number of measured variables (reduced sleep latency, improved sleep efficiency, reduced WASO, and prolonged sleep time) during PSG and improved CPAP titrations with fewer residual events and fewer incomplete titrations [89]. As increased awareness of sleep-disordered breathing has generated a growing demand for PSG, results of this trial could significantly reduce the total number of studies required to be performed, resulting in greater efficiency for sleep laboratories.

9 Safety Profile

Zopiclone and eszopiclone's safety and tolerability profile has been evaluated in clinical studies, and assessments of clinical laboratory tests (including hematology, blood chemistry, urinalysis), physical examinations, vital signs, and EKGs revealed no evidence of a significant safety risk associated with administration of either hypnotic agent. Overall, both zopiclone and eszopiclone were well tolerated in patients with insomnia during clinical trials, as inpatients and outpatients, and during short-term and longer-term exposure.

The most common adverse event noted with zopiclone in clinical studies was taste alteration or dysgeusia (a bitter or metallic aftertaste), reported in 15–30% of treated patients [39, 90, 91]. The bitter taste is related to salivary excretion of the hypnotic and corresponds to saliva levels, although in most patients the occurrence of the unpleasant taste did not prevent continued use of the hypnotic. Zopiclone at doses up to 5 mg/day in elderly patients was generally well tolerated and produced no serious or unexpected adverse events, and no patients receiving the hypnotic had to discontinue treatment due to adverse events [92]. In a large postmarketing surveillance study of 20,513 patients, the overall incidence of adverse events did not differ with age; 9.1% in patients 15–64 years old, 9.5% in patients 65–79 years old, and 9.9% in patients >80 years old [68]. No serious adverse events were reported with zopiclone 7.5 mg/day, and the most frequently reported events were

bitter taste (3.6%), dry mouth (1.6%), difficulty arising in the morning (1.3%), and daytime sleepiness (0.5%). All were judged by patients to be mild in magnitude. The incidence of treatment discontinuation due to adverse events was 2.8 and 24% of patients (0.9% of the total study population) who experienced bitter aftertaste discontinued treatment for this reason. Anterograde amnesia, commonly reported with use of benzodiazepines, was reported by <0.1% of patients in prescription-event monitoring studies of zopiclone. Other rarely occurring gastrointestinal adverse effects included nausea, vomiting, epigastric pain, diarrhea, or constipation. Data from another large clinical trial with zopiclone are broadly consistent with those from the postmarketing trial. The bitter aftertaste with zopiclone was reported with a frequency of 6.7%, compared to 0.7% in the placebo-treated group, and 0.8% of zopiclone-treated patients withdrew from the study for this reason. The taste disturbance associated with zopiclone was the only adverse event that distinguished the tolerability profile of the hypnotic from that of flunitrazepam and triazolam [93]. Zopiclone administration has been noted to produce minimal or no respiratory depression in patients with respiratory disorders. Likewise, there have been no reports of changes in respiratory rate, pulse, blood pressure, or laboratory parameters noted following zopiclone use [45].

As with zopiclone, unpleasant and bitter taste was the most commonly reported adverse event noted to occur in adults administered eszopiclone across the dose range from 1 mg (8%) [67] to 3 mg (34%) [51]. In addition to unpleasant taste, other adverse events that suggest a dose–response relationship in pooled data in elderly patients taking eszopiclone 1 mg or 2 mg included pain and dry mouth [52, 67]. A recent study noted that although no meaningful relationships were found between the frequency or the intensity of the taste disturbance and age, body mass index or phenyl thiocarbamide taste sensitivity; dysgeusia was more intense and long lasting in females than in males, stronger in the morning than in the evening, and positively correlated with the hypnotic's plasma and saliva levels [94]. Adverse events in nonelderly patients administered eszopiclone 2 mg or 3 mg suggest a dose–response relationship with viral infection, dry mouth, dizziness, hallucinations, infection, rash and unpleasant taste, with unpleasant taste having the strongest dose–response relationship [51]. The most frequently reported adverse events in two 6-month trials were unpleasant taste (20% and 26%), infection (16% and 17%), headache (15% and 20%), nausea (11% and not reported), pain (9% and 11%), and somnolence (both 9%) [64, 66]. Adverse events reported during the clinical trials in at least 2% of the study population were generally mild to moderate in severity and transient in duration, resulting in minimal study subjects discontinuing treatment.

During the two 2-week trials in elderly patients, the overall incidence of adverse events observed between treatment groups and placebo was similar. The most commonly reported adverse events reported with eszopiclone 1 mg and eszopiclone 2 mg compared to placebo were: headache (15.3%, 15.2%, 15.0%), unpleasant taste (8.3%, 11.4–12.5%, 0–1.3%), somnolence (6.9%, 3.8–6.6%, 5.5–8.8%), and dyspepsia (5.6%, 1.3%, 2.5%). No reported adverse events related to accidental falls, anterograde amnesia, or hallucinations were noted. Likewise, in the longer-term 12 week trial with elderly patients, the number of patients with adverse effects was

59.3% in the eszopiclone group compared with 50.5% in the placebo group [70]. Other than headache, nasopharyngitis, and unpleasant taste, no other adverse event was reported by >5% of patients in either treatment group, although unpleasant taste was significantly different from placebo. Generally, the adverse effect profile in the very elderly (>75 years) was similar to the elderly (<75 years). In the >75 years old age group, asthenia (fatigue) was higher while unpleasant taste was somewhat lower; both expected changes in the elderly.

The rate of discontinuation due to adverse events did not differ between eszopiclone and placebo groups in trials of 6 weeks' duration or less [69] and in one 6-month trial where 8.6% of eszopiclone-treated patients and 7.5% of placebo-treated patients discontinued therapy due to an adverse event [66]. However, in the 6-month trial [64], high dropout rates occurred in both the active treatment (36.5%) and placebo groups (43.4%). The rate of discontinuation due to adverse events was 12.8% in the eszopiclone group and 7.1% in the placebo group, and the most common reasons cited for discontinuation were: somnolence (2.2% for eszopiclone vs. 1.5% for placebo), depression (2.0% vs. 0%), unpleasant taste (1.7% vs. 0.5%), headache (0% vs. 2.0%), asthenia (1.0% vs. 1.5%), and insomnia (0% vs. 1.5%), respectively.

The adverse effect profile of eszopiclone in the trials in patients with comorbid insomnia was generally similar to that noted in patients with primary insomnia. The most frequently reported adverse events in patients treated with eszopiclone 3 mg compared to placebo were unpleasant taste (18–27% vs. 0–4%), headache (10–19% vs. 8–15%), nausea (10–13% vs. 13–15%), dry mouth (4–16% vs. 1–9%), and somnolence (4–11% vs. 3–7%).

In the crossover study, which assessed the effects of eszopiclone on measures of respiration and sleep using PSG readings in patients with OSAS, eszopiclone was well tolerated with no deaths, serious adverse events, or adverse events leading to discontinuation during treatment reported [88]. No adverse events related to drug class effects, such as anxiety, confusion, hallucinations, or insomnia were reported during the study period. Eszopiclone treatment did not adversely affect any sleep apnea parameter including apnea/hypopnea and oxygen saturation measurements. Additionally, derived QT_C values for each patient, which were calculated from the RR interval and the QT interval using Bazett's formula: $QT_{C-B} = QT/(RR/100 \text{ ms})^{1/2}$, noted that during the study, no QT_{C-B} value exceeded 500ms during any treatment period at any time point. Unpleasant taste occurred in six of the 21 patients (28.6%) treated with eszopiclone.

In the trial that incorporated zolpidem as an active control, the adverse events observed with eszopiclone 3 mg, zolpidem 10 mg, and placebo, respectively, were dizziness (4.7% vs. 10.9% vs. 4.8%), somnolence (4.7%, 9.4%, 0%), headache (9.4%, 9.4%, 9.5%), and hallucinations (0%, 4.7%, 0%). Unpleasant taste was the only adverse event that was observed with a greater frequency in the eszopiclone group compared to zolpidem or placebo (7.8%, 0%, 1.6%) [95].

One report exists of a patient developing transient visual and auditory hallucinations following a week of eszopiclone 3 mg treatment [96]. The patient had a previous history for acute psychotic symptoms and received eszopiclone to be taken

at 9 a.m.; however, his sleep pattern was very erratic during the week preceding his hospitalization, and he was averaging only 4 h of sleep per day. Eszopiclone should not be taken if the patient is unable to get at least 8 h of sleep, and the safety of NBRAs for treating shift-work type of circadian rhythm sleep disorders has not been documented. Hence, more research needs to be undertaken to ascertain the safety of NBRAs in circadian rhythm sleep disorder, especially the shift-work type.

9.1 Residual Effects and Daytime Psychomotor Function

Pharmacodynamic and patient-reported assessments were utilized across the clinical development program to evaluate zopiclone and eszopiclone's impact on cognitive, memory, and psychomotor effects. Generally, assessments of psychomotor performance following zopiclone administration have been varied. Most studies have shown that zopiclone 7.5 mg administered at night did not produce significant next-day residual effects on psychomotor performance in healthy volunteers and insomniac patients [45, 97]. However in some studies, single doses of zopiclone 7.5 mg caused impairment in coordination test [98] and in performance of skilled tasks [39, 99] the following morning. Zopiclone doses >10 mg are noted to cause psychomotor impairment more frequently [33, 39] as seen by significant next-day variations in the mean scores of the complex reaction time test [100]. A review assessing psychomotor performance noted few reports of residual effects following zopiclone administration, with the majority being of small magnitude and rarely persisting beyond 12 h [101]. Multiple outpatient population studies conducted in the early clinical development of zopiclone have noted no psychomotor performance decrements following chronic administration [102].

Zopiclone, because of its shorter half-life, produced less daytime residual sedation and impairment in next-day psychomotor functioning than long-acting benzodiazepines, flurazepam, flunitrazepam, and nitrazepam [33, 45]. Residual effects were similar to, or less than, those observed with more rapidly eliminated hypnotics, such as triazolam and temazepam [45, 90], and on the basis of the digit substitution test, 10 mg of zopiclone was equivalent to 0.5 mg triazolam [103].

Generally, impairments in psychomotor, cognitive, or memory function were infrequent in patients who received eszopiclone [69]. Eszopiclone at a dose range from 1 to 3 mg did not demonstrate psychomotor impairment in patients following daytime dosing of the hypnotic, with mean DSST and Stanford Sleepiness Scale (SSS) scores returning to baseline at 6 h postdose [34]. In the transient insomnia study [53], no impairment was observed on psychomotor performance, as measured by DSST scores, for any treatment group (eszopiclone 1 mg, 2 mg, 3 mg, 3.5 mg), and self-reported morning sleepiness scores were significantly better for eszopiclone 3 mg and 3.5 mg compared with placebo. In the 6-week trial [51], next-day residual effects evaluated via DSST administered approximately 1–1.5 h after awakening, and morning patient-reported daytime function, noted no differences

between treatment groups at baseline relative to placebo, and no decrements in scores from baseline at any time point.

9.2 *Rebound Insomnia and Withdrawal Effects*

Rebound insomnia, defined as worsening of sleep parameters relative to baseline following discontinuation of treatment, has been observed with benzodiazepines, especially short-acting benzodiazepines, in a dose- and time-dependent manner, occurring after only 1–2 weeks of treatment [104]. Most studies note a lack of rebound insomnia on discontinuation of the Z-hypnotics [105].

Rebound effects due to zopiclone withdrawal are considerably less frequent than with benzodiazepines and when they do occur, they generally resolve after 1 or 2 days following withdrawal [106–108]. Most studies that included a withdrawal phase reported no statistically significant rebound effects following zopiclone withdrawal [90], although some studies did note significant rebound insomnia after withdrawal of zopiclone [109, 110] even when zopiclone was given for short periods (5–14 days) to healthy volunteers and insomniac patients [33, 111]. A postmarketing study also noted that one single case of rebound insomnia was reported out of 2,542 patients who received zopiclone 7.5 mg for 28 days [90]. A review of 25 studies found no evidence of rebound insomnia as assessed by standard sleep variables in patients who received zopiclone 7.5 mg, while rebound insomnia was noted after withdrawal of flurazepam, nitrazepam, and triazolam. Withdrawal effects were observed in only 2.7% of cases in the same review [112]. Most adverse events occurring following zopiclone withdrawal were similar to those observed following withdrawal from placebo. Abrupt discontinuation of zopiclone following prolonged use, and/or misuse in excessive doses, has been noted to cause symptoms of withdrawal ranging from anxiety, tachycardia, tremor, sweating, rebound insomnia, flushes, palpitations, derealization, seizures, and delirium [113–115]. Zopiclone has also been shown to ease the gradual withdrawal from long-term use of long-acting benzodiazepines and may be used as a facilitator agent during the gradual tapering of the hypnotics [116]. In patients with generalized anxiety disorder and comorbid insomnia, rebound anxiety was the most frequently reported adverse event following zopiclone withdrawal, although the incidence was less than that observed following triazolam withdrawal (1.1% vs. 13%, respectively) [39].

Most controlled studies have noted no evidence of rebound insomnia after discontinuation of eszopiclone [117]. No reports of seizures, hallucinations, or perceptual-disturbance events that are commonly reported as withdrawal symptoms following discontinuation of sedative–hypnotic agents were reported upon termination of eszopiclone therapy. However, one study found rebound insomnia on the first night in patients discontinuing eszopiclone 2 mg, and diminished sleep efficiency on the first night in patients discontinuing eszopiclone 3 mg [118]. Likewise, 48 h following discontinuation of eszopiclone and initiation of placebo treatment,

adverse events at a reported incidence of $\leq 2\%$ occurred, including anxiety, abnormal dreams, nausea, and upset stomach [18]. In the 26-week trial with adults with chronic insomnia [66], there was no significant difference between eszopiclone 3 mg and the placebo groups for the total score outcome of the benzodiazepine withdrawal symptom questionnaire (BWSQ), a subjective assessment of symptoms the subject may be experiencing after discontinuing study medication. Although no evidence of serious withdrawal symptoms during clinical trial experience were reported, these events may need to be evaluated further to see if dosage reductions are necessary upon treatment discontinuation [119].

In patients who received eszopiclone with fluoxetine for 8 weeks in managing insomnia with coexisting depression, no evidence of benzodiazepine withdrawal adverse events, rebound insomnia, rebound depression, or tolerance were observed following discontinuation of the hypnotic [75]. Likewise, insomnia patients with GAD who received eszopiclone with escitalopram for 8 weeks reported no evidence of rebound insomnia and no evidence of development of tolerance following eszopiclone discontinuation over a 2-week period [79].

Postmarketing experience based on 1.39 million-person years exposure indicates that only 0.18% of total cases of tolerance are reported, 0.19% of withdrawal syndromes have been reported, and 0.33% cases of rebound effect have been documented [24].

9.3 Tolerance, Abuse, and Dependence

Studies have shown that zopiclone maintained its hypnotic efficacy for 2 [120] to 3 weeks [110, 121] and 6 weeks [122] based on subjective assessments of sleep parameters. Likewise, polysomnographic recordings did not detect tolerance following 8 [123] or 17 weeks [63] of zopiclone therapy in chronic insomniac patients.

No evidence of development of tolerance was noted upon discontinuation of eszopiclone, after 6 weeks [51], 12 weeks [70, 71], 6 months [64, 66], or 12 months of nightly administration [65]. No evidence of eszopiclone abuse was observed during the clinical trials. However, it shares some of the pharmacological properties of benzodiazepines and, as a schedule IV controlled substance in the United States, is subject to restrictions and monitoring for possible physical and psychological dependence [19]. Reports of dependence have been very low (0.11%) based on postmarketing experience with eszopiclone [24].

Several reports of drug abuse with zopiclone have been reported and the abuse potential of zopiclone was found to be similar to that of zolpidem. Most cases of zopiclone misuse or dependence were reported in patients aged between 16 and 60 years [114] who suffered from preexisting addiction or chemical abuse, or from underlying psychiatric conditions [115]. A European study noted that the rate of reported misuse with NBRAs was about one-third the rate reported for benzodiazepine misuse [114], and it has been hypothesized that the lower rate of misuse may reflect less binding to the α -2 unit, which may play a role in dependence.

The incidence of zopiclone dependence in patients who are long-term users of the hypnotic was assessed in a 6-month follow-up cohort study in combat veterans with posttraumatic stress disorder (PTSD). It was noted that the patients did not use zopiclone at a dose or frequency greater than that prescribed, and patients reported similar follow-up tranquilizer dependence scores as those reported at baseline [124].

Reports on zopiclone dependence point out that polydrug users may use zopiclone for producing euphoria. A survey of polydrug abusers in a methadone clinic noted that most individuals who report drug abuse with zopiclone initially used the hypnotic for sedation but later developed tolerance and subsequently used higher doses of oral zopiclone (90–380 mg daily) [125]. In France, for example, zopiclone is in the “top ten” of false prescriptions; 4% of users obtain zopiclone from other people or from dealers, and 15% of regular users take the hypnotic for another goal than hypnotic use, mainly for euphoria, stimulation, and even to disinhibit an anxiolytic effect [126]. Hence, it may be considered a substance of abuse. Self-administration of zopiclone by rhesus monkeys was reported to occur “relatively frequently” by the intravenous route [127], suggesting a high risk of abuse potential. Abuse of intravenous zopiclone in humans is also cited [128].

A study evaluating the abuse liability of subjects with known histories of benzodiazepine abuse noted that eszopiclone at doses of 6 mg and 12 mg produced euphoric effects similar to those of diazepam 20 mg. Both eszopiclone and diazepam caused a dose-related increase in reports of amnesia and hallucinations, at doses twofold or greater than the maximum recommended doses [18].

10 Conclusion

Both zopiclone and eszopiclone are cyclopyrrolone hypnotics chemically unrelated to the benzodiazepines. Their hypnotic effect is believed to result from selective binding activity for GABA_A receptor subtypes, for receptor-associated ion chloride channels and ion current. Zopiclone is a racemic mixture of two stereoisomers, and studies have noted that S-zopiclone (eszopiclone) is more active than R-zopiclone at the benzodiazepine receptor complex and hence is responsible for most of the hypnotic activity of the racemic compound.

Both zopiclone and eszopiclone are well established as effective and well tolerated hypnotics. Their $t_{1/2}$ of ~5–6 h is particularly beneficial in elderly patients who frequently complain about sleep initiation and sleep maintenance, including frequent nocturnal awakenings, inability to go back to sleep after awakening, fragmented sleep, early morning awakenings, and difficulty remaining awake during the day. Zopiclone has at least similar efficacy to the majority of benzodiazepines and results in comparable improvements in latency of onset, duration, and quality of sleep. Although zopiclone has been found to be well tolerated at higher doses, the use of 7.5 mg has been found to be the optimum dose for most patients. In healthy subjects, therapeutic doses of zopiclone exert no or few negative influences

on next-day cognitive functions; however, higher than recommended doses can be detrimental to cognitive performance.

Across all the efficacy clinical trials, eszopiclone 2 mg and 3 mg consistently demonstrated statistically significant improvement of the primary variable, latency to persistent sleep, measured either objectively or subjectively in both the short and in the long-term trials. Efficacy was demonstrated also with regard to the secondary efficacy measures, which assessed sleep maintenance, sleep quality, and daytime functioning, although only the 3 mg dose consistently produced significant improvements in sleep maintenance parameters. Throughout each month of the long-term studies, results were sustained for up to 12 months. Significant improvements in next-day effects, including daytime alertness, ability to concentrate and function, and sense of physical well-being have also been reported.

The increased effectiveness of eszopiclone at the α_2 and α_3 and a decreased effectiveness at the α_1 and α_5 GABA-A receptors would result in decreased memory effects and beneficial effects with respect to anxiety, depression, and pain. Clinical trials have demonstrated efficacy with eszopiclone in patients with comorbid insomnia with respect to reductions in anxiety, depression, and pain that are not apparent for racemic zopiclone. Clinically, the data from these trials suggest that, in addition to the comorbid condition that may exist, the insomnia needs to be treated directly to maximize patient outcomes.

In clinical trials, the tolerability profile of zopiclone and eszopiclone was similar to placebo with the exception of bitter taste, which occurred more frequently with the hypnotics. Both hypnotics have demonstrated no evidence of tolerance or rebound insomnia following discontinuation. A history of alcohol or drug abuse, or psychiatric disorders may increase a patient's risk for abuse of, and dependence on, sedative-hypnotic agents; hence, cautioned use of zopiclone or eszopiclone is advised in such patients.

Because of a lack of head to head studies of eszopiclone with short-acting benzodiazepines, a direct comparison with these sedative-hypnotics is not available to delineate differences between these agents. Racemic zopiclone has been evaluated extensively with various benzodiazepine hypnotics and found to have a similar efficacy and adverse event profile to the benzodiazepines. Future comparative trials evaluating eszopiclone with other NBRA sedative-hypnotics, including racemic zopiclone, need to be undertaken to assess its relative efficacy and tolerability.

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Indiplon

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Abstract Indiplon is an investigational hypnotic that has been evaluated in efficacy and safety studies as a possible treatment for insomnia. It is a positive allosteric modulator of GABA responses at the GABA_A receptor complex and has selectivity for receptors containing the $\alpha 1$ subunit subtype. The compound's short elimination half-life has allowed the development of immediate-release and modified-release formulations. Clinical trials have been conducted with populations of adult and elderly insomnia subjects. Safety assessments suggest that indiplon is well-tolerated in both groups. The immediate-release capsule has been shown to be beneficial for sleep onset at bedtime or during middle-of-the-night awakenings, while the modified-release formulation has positive efficacy for sleep onset and sleep maintenance. At present, neither formulation has been approved by the US Food and Drug Administration or regulatory organizations in other countries.

1 Introduction

A wide range of medications and other sedating substances are employed in the attempt to help patients with insomnia fall asleep and remain asleep. Among these compounds are FDA-approved medications, sedating medications without specific indications for the treatment of insomnia, over-the-counter (OTC) antihistamines, and assorted unregulated herbal preparations and dietary supplements, such as valerian and melatonin. Beginning in the 1960s, benzodiazepine compounds were first available, and within a decade, benzodiazepine hypnotics became the mainstay of insomnia pharmacotherapy. These have included estazolam, flunitrazepam, flurazepam, loprazolam, lormetazepam, nitrazepam, quazepam, temazepam, and

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triazolam. Although benzodiazepine hypnotics continue to be available, over the past two decades, they have played a less prominent role in insomnia treatment. These hypnotics that incorporate a benzodiazepine structure have varying pharmacologic properties that significantly influence the hypnotic duration of action. Further, assorted other benzodiazepine compounds that are neither indicated nor licensed for the treatment of insomnia may be prescribed for this purpose. Among the potential concerns that have limited the use of benzodiazepines in the treatment of insomnia have been tolerance, withdrawal, abuse liability, labeling restrictions, and the possibility for residual daytime effects with the longer half-life compounds [1].

The newer generation benzodiazepine receptor agonists that do not incorporate a benzodiazepine chemical structure initially became available during the 1980s and 1990s, and new variations of these compounds continue to be developed and marketed. Among the approved or licensed nonbenzodiazepine compounds are eszopiclone (Lunesta), zaleplon (Sonata), zolpidem (Stilnox, Ambien, Ambien CR, Myslee, Edluar, Zolpimist), and zopiclone (Imovane). These nonbenzodiazepine hypnotics generally have greater selectivity at the pentameric GABA_A receptor complex for the $\alpha 1$ subtype of the α subunit. This selectivity may contribute to more favorable safety and tolerability profiles among these medications. Additionally, the nonbenzodiazepine compounds generally have comparatively shorter elimination half-lives that reduce the potential for next-day residual effects and therefore contribute to their safety and tolerability. Indiplon, which has been investigated in both immediate- and modified-release formulations for the treatment of insomnia, is one of the recently investigated benzodiazepine receptor agonist hypnotics that do not incorporate the benzodiazepine structure.

The development of indiplon was initiated in the 1980s at the American Cyanamid Lederle Laboratories as CL 285,489, which also was the case for zaleplon (Sonata, CL 284,846). In 1994, American Cyanamid was acquired by Wyeth-Ayerst and its parent company, American Home Products. In 1998, the worldwide exclusive licenses of several compounds, including the future indiplon, were acquired by DOV Pharmaceutical, Inc., which was founded in 1995 by former Lederle Laboratories researchers involved in the development of these compounds. Subsequently in 1998, CL 285,489 was sublicensed on an exclusive worldwide basis to Neurocrine Biosciences, a company in San Diego, CA, founded in 1992. Neurocrine began Phase I studies to develop the compound (NBI-34060) as a hypnotic and, in 2001, initiated Phase III clinical trials in the US and Europe. In 2002, a new composition of matter US patent (6,399,621) with an expiration date of 2020 was secured. Neurocrine also secured a US patent (6,485,746) for a controlled-release formulation of NBI-34060 in 2002 [2]. In 2002, Neurocrine entered into an agreement with Pfizer regarding the development and commercialization of indiplon; however, in 2006, the agreement was terminated in the context of delays in the approval process. In 2004, Neurocrine acquired Wyeth's financial interest in indiplon, leaving only DOV to receive future royalty and milestone payments.

Indiplon has been investigated in two formulations – a short-acting immediate-release (IR) capsule and a longer-acting modified-release (MR) tablet. This is possible due to the compound's short elimination half-life. The MR formulation

incorporates both immediate and delayed release for a more sustained sleep-promoting effect. The rapid decrease in the medication serum level resulting from the short half-life should minimize the risk of next-day residual sedation. The pharmacokinetics of the indiplon formulations suggest that bedtime use of the MR tablet could enhance sleep throughout the night, while the short-acting IR capsule could allow the flexibility for dosing at bedtime or with middle-of-the-night awakenings.

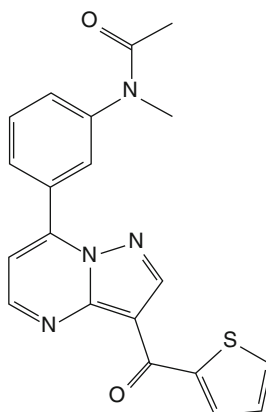
At the time of this writing, indiplon remains an investigational compound, and it is unclear whether it will be approved and commercialized in the US or in other countries. Neurocrine initially applied to the US Food and Drug Administration (FDA) for approval of 5 and 10 mg dose indiplon-IR capsules and for an indiplon-MR 15 mg tablet. In May 2006, the FDA issued complete responses, which included an approvable letter for the indiplon-IR doses and a nonapprovable letter for indiplon-MR [3].

The FDA nonapprovable response pertaining to the indiplon-MR tablets questioned the sleep maintenance efficacy at that dose, as most of the submitted studies had been performed at higher doses. The FDA also requested the reanalysis of safety and efficacy data. In a follow up meeting, the FDA requested additional long-term safety and efficacy studies in adults with the 15 mg dose and the development of a separate dose for elderly patients [3]. There has been no resubmission to the FDA for the indiplon-MR tablets.

With regard to the indiplon-IR doses, the FDA requested the reanalysis of data to support the sleep initiation and middle-of-the-night dosing indications, reexamination of safety data, and supplemental pharmacokinetic studies related to food effects. Neurocrine complied with these requests and the FDA responded to the submitted data in December 2007 stating that the indiplon doses remained approvable, but that now there would be new requirements before indiplon would be approved. Specifically, the FDA requested a new objective and subjective clinical trial in elderly subjects, a new safety study assessing adverse event rates in indiplon compared with a marketed product, and a preclinical study evaluating indiplon given during the third trimester of pregnancy [3]. The projected expenses and further time delays associated with these large-scale studies apparently have led to the discontinuation of the development of indiplon in the US. However, Neurocrine has entered into an agreement with Dainippon Sumitomo (which recently purchased Sepracor, the developer and manufacturer of eszopiclone) for the development and commercialization of indiplon in Japan.

2 Indiplon Chemistry

Indiplon (NBI-34060) is a pyrazolopyrimidine compound. It has the molecular formula $C_{20}H_{16}N_4O_2S$. The chemical name is *N*-methyl-*N*-[3-[3-(2-thienylcarbonyl)-pyrazolo[1,5- α]pyrimidin-7-yl]phenyl]acetamide [2]. The chemical structure of indiplon is shown in Fig. 1.

Fig. 1 Chemical structure of indiplon

3 Pharmacodynamic Characteristics

Indiplon is a high-affinity, positive allosteric modulator of GABA_A responses with a preference for $\alpha 1$ subunit-containing GABA_A receptors [4]. It shares certain key pharmacodynamic features with other recent benzodiazepine receptor agonist hypnotics described as nonbenzodiazepines, such as eszopiclone, zaleplon, zolpidem, and zopiclone. However, these medications vary in their receptor affinity and subunit subtype ($\alpha 1$) selectivity, as well as in their pharmacokinetic features.

The results of *in vitro* experiments characterizing fundamental pharmacological properties of indiplon were published by Neurocrine researchers in 2004 [5]. Reversible binding and high affinity for the GABA_A receptor benzodiazepine site, as shown by a K_i value about 50 and 10 times lower than zaleplon and zolpidem, respectively, were demonstrated with rat brain membrane radioligand binding studies. Preferential labeling of $\alpha 1$ subunits containing GABA_A receptors was evident in the binding pattern. Indiplon was shown to have full agonist activity through potentiation of GABA_A with GABA shift experiments and patch-clamp recordings employing cultured rat cortical neurons. The selectivity for GABA_A receptors containing an $\alpha 1$ subunit compared with the $\alpha 2$, $\alpha 3$, or $\alpha 5$ subunits was estimated to be 10-fold [6]. Neurocrine researchers concluded that indiplon has greater potency than zolpidem and zaleplon in potentiating the GABA-induced chloride current in rat cerebellar membrane-cultured neurons due to the inhibition of [3H]Ro 15-1788 binding with an affinity of 1.5 nM [5].

Rodent *in vivo* indiplon oral administration studies demonstrating pharmacodynamic properties also were reported by Neurocrine researchers in 2004 [4]. Experiments with mice and rats showed dose-dependent behavior changes typical of sedative-hypnotic compounds and consistent with those having $\alpha 1$ subunit selectivity. Locomotor activity, passive avoidance retention, and open field test assessments were performed in the mouse studies, while locomotor activity, rotarod latency, vigilance, delayed nonmatch to sample, and a Vogel test were assessed

with the rat studies. Zaleplon and zolpidem comparisons also were incorporated into these experiments.

Human quantitative EEG analysis was conducted as a general pharmacodynamic representation of the potential value of a compound as a hypnotic. In one such study, 30 healthy young males were assessed after they were given indiplon 10, 30, 45 mg, or placebo for 14 days [7]. The researchers found a dose-related decrease in the cortical amplitude and occipital α -1 wave and a frontal cortical beta wave amplitude increase consistent with hypnotic medications. The results in this study suggested an absence of pharmacodynamic and pharmacokinetic tolerance. Further, the quantitative EEG studies showed that a relatively low indiplon serum level of 5 ng/ml was a sufficient threshold for these pharmacodynamic changes characteristic of a sedative property.

4 Pharmacokinetics Characteristics

The pharmacokinetic properties of indiplon have been investigated in both in vitro and in vivo studies in animals and humans. Two major metabolites with significantly different metabolic pathways were found with pooled human liver microsomes studies. *N*-desmethyl-indiplon formation, which represents 60% of the metabolites, primarily utilizes the CYP3A4/5 isoenzymes, although there is a minor contribution from the CYP1A2 isoenzyme. Organophosphate-sensitive microsomal carboxylesterases catalyze *N*-deacetyl-indiplon accounting for 40% of the metabolites. Accordingly, there is low potential for hepatic isoenzyme inhibition by indiplon. These findings suggest that there is minimal risk for pharmacokinetic interactions between indiplon and coadministered drugs [8, 9]. Further, healthy males given a combination of indiplon (10 mg) and alcohol (0.7 mg/ml) revealed no significant pharmacokinetic interactions [10].

A series of human pharmacokinetic investigations have demonstrated the absence of a gender effect [11], no relationship with body weight [11], and no significant differences between young and old subjects [12]. Pharmacokinetic studies of indiplon with oral dosing in mice and rats showed that it reached a maximum concentration at 30 min and had an elimination half life of 1 h [4]. A gender comparison study with healthy young males and females demonstrated mean maximum blood concentrations of indiplon at less than 1 h following a 15 mg dose and mean elimination half-lives of less than 2 h [11]. A study comparing young and elderly subjects found a mean time to reach maximum concentration on night 1 following an indiplon 15 mg dose was 2.3 h for the young and 2.7 h for the elderly subjects. The respective mean elimination half-lives for the young and elderly subjects were 1.5 and 1.8 h [12].

An absence of pharmacokinetic tolerance following repeated doses was demonstrated in a study of 30 healthy young male subjects who were given indiplon 10, 30, 45 mg, or placebo for 14 consecutive days [7]. The pharmacokinetic characteristics were similar for day 1 and day 14.

The pharmacokinetic investigations have shown that indiplon is rapidly absorbed and eliminated with minimal effect due to gender, age, and body weight. There is minimal risk of pharmacokinetic interaction with coadministered medications. These key pharmacokinetic properties make indiplon an excellent candidate for the modification to a sustained-release formulation. The rapid blood level decline minimizes the potential for undesired residual sedation or impairment the morning following bedtime dosing.

5 Clinical Efficacy: Indiplon Immediate-Release

The efficacy of indiplon-IR was investigated in a series of single and multiple dose controlled clinical trials. In a single night clinical trial, 228 healthy adults with no history of insomnia were given a solution of indiplon 15, 30 mg, or placebo in an environment intended to create transient insomnia [13]. The transient insomnia model incorporated the expected sleep disturbing first night effect in a sleep laboratory along with a bedtime phase advanced by 2 h. Both objective and subjective assessments for sleep onset with the indiplon group were significantly better compared with the placebo subjects. In this study, the total sleep time was not significantly affected by the medication. Another transient insomnia study included 593 healthy adults given indiplon-IR 10, 20 mg, or placebo [14]. The two indiplon dose groups had better subjective and objective sleep onset, total sleep time, sleep efficiency, and sleep quality compared with the placebo group.

The efficacy of indiplon-IR was assessed in a 35-day treatment study of 194 chronic insomnia subjects (DSM-IV primary insomnia criteria with at least 3 months duration) given placebo, 10, or 20 mg doses [15]. The study design included subjective assessments in addition to two consecutive polysomnographic recording nights at the beginning, middle, and end of the treatment period, and also during the first two nights of the single-blind discontinuation period. Compared with placebo, the objectively determined latency to persistent sleep was significantly better for both indiplon-IR doses at all time points. There was no objective or subjective evidence of withdrawal or rebound insomnia on abrupt, single-blind drug discontinuation.

In a sleep laboratory study with a four-period crossover design, 42 elderly chronic insomnia (DSM-IV primary insomnia) subjects (mean age 70 years; range 65–82 years) were given indiplon-IR 5, 10, 20 mg, and placebo [16]. Compared with the placebo nights, each of the indiplon doses was associated with significantly better objective and subjective sleep onset latency, while the two higher doses additionally resulted in longer total sleep times.

In an outpatient study of 358 older adults with primary insomnia, the subjects were given indiplon-IR 5, 10 mg, or placebo nightly for 2 weeks [17]. During both weeks, the groups taking the indiplon doses had significantly shorter reported latency to sleep onset compared with the placebo group. The subjectively reported total sleep time, number of awakenings, and sleep quality were significantly

improved during the 2 weeks. The 10 mg indiplon-IR group also had a significant reduction in the wake time after sleep onset.

A 3-month outpatient study of 702 adult chronic insomnia subjects assessed the long-term efficacy of indiplon-IR with a comparison of nightly 10 and 20 mg doses with placebo [18]. Compared with the placebo subjects, the subjective latency to sleep onset, total sleep time, wake time after sleep onset, number of awakenings, and sleep quality all were significantly better with the two indiplon doses at all time points. Upon abrupt single-blind discontinuation, there was no evidence of withdrawal symptoms. Continued efficacy with improved time to sleep onset was present in a subset of the study population that remained on nightly doses of indiplon-IR for 6 months.

Adults with chronic insomnia ($N = 260$) characterized by prolonged nighttime awakenings with difficulty returning to sleep were recruited to participate in a 4-week subjective study with indiplon-IR doses given during middle-of-the-night awakenings [19]. The subjects were randomized to groups taking as-needed doses of indiplon-IR 10, 20 mg, or placebo during the night when at least 4 h remained available for sleep. The study's primary endpoint was the postdose subjective latency to sleep onset. Both indiplon doses were associated with significantly shorter sleep-onset values compared with placebo. The 4-week average postdose subjective total sleep time additionally was significantly improved relative to placebo. A visual analog scale assessment demonstrated no evidence of next-day sleepiness.

6 Clinical Efficacy: Indiplon Modified-Release

The modified-release indiplon formulation efficacy studies have been done primarily with groups of chronic insomnia subjects. One exception is a pharmacokinetics study with healthy young male volunteers during which the subjects had repeated venipunctures throughout the study night to assess the plasma drug concentrations [20]. A 40 mg dose of indiplon-MR or placebo was given to 36 subjects at 10 p.m. The venipuncture model of sleep initiation and maintenance insomnia was effective in disrupting normal sleep patterns. Compared to the placebo subjects, the indiplon-MR group reported significantly better sleep onset, total sleep time, and sleep quality.

A dose-response polysomnography study of indiplon-MR examined the efficacy of the drug in 47 chronic insomnia subjects with sleep maintenance difficulty given placebo and indiplon-MR 20, 30, 35, and 40 mg in a crossover schedule [21]. The three highest indiplon-MR doses resulted in significantly better sleep efficiency compared with placebo dose. The latency to persistent sleep and number of awakenings were significantly better for all of the indiplon-MR doses compared with the placebo nights.

In a subjective outpatient study, adult chronic insomnia subjects ($N = 211$) in a single-blind fashion were given a placebo for 2 weeks followed by nightly doses of either indiplon-MR 30 mg or placebo [22]. During the 2 treatment weeks, the subjects in the indiplon-MR group reported significantly better total sleep time,

wake after sleep onset, number of awakenings, latency to sleep onset, and sleep quality ratings relative to the placebo group.

In a 4-week treatment period outpatient study, chronic insomnia subjects with sleep maintenance complaints ($N = 248$) were given nightly doses of either indiplon-MR 15 mg or placebo. The indiplon-MR group reported significantly better outcomes in all of the study endpoints, including subjective total sleep time where there was an average of 1 h more sleep per night [23].

Sixty older adult subjects (ages 65–75 years) meeting criteria for chronic sleep maintenance insomnia, as well as polysomnographic screening criteria, participated in a sleep laboratory assessment of indiplon-MR. The subjects were randomly divided into groups dosed for two consecutive nights with placebo or indiplon-MR 10, 20, 30, or 35 mg [24]. At all indiplon-MR doses, there was a significant decrease in the objective latency to persistent sleep, and at the three higher doses, there was a significant decrease in the wake after sleep onset (WASO) compared with the placebo subjects.

An outpatient indiplon-MR study of 229 older adult chronic insomnia subjects (ages 65–85 years) investigated the subjective evaluation of the efficacy and tolerability of indiplon-MR during a 2-week treatment period [24]. The subjects were randomly assigned to groups taking nightly doses of indiplon-MR 15 mg or placebo. The indiplon-MR group reported significant improvement compared with placebo for the assessments of total sleep time, time to sleep onset, wake time after sleep onset, number of awakenings after sleep onset, and total wake time at both study weeks. There was no evidence of next-day sleepiness or impairment in the indiplon-MR group.

A 3-month outpatient study of indiplon-MR was performed with 740 adult chronic insomnia subjects [25]. The participants were randomly divided into groups dosed with nightly indiplon-MR 20, 30 mg, or placebo. The two indiplon-MR dose groups reported significantly better outcomes for all study endpoints at all time points compared with the placebo group. The outcomes included subjective total sleep time, latency to sleep onset, total wake time, wake time after sleep onset, number of awakenings after sleep onset, and sleep quality.

Pooled data from four crossover Phase 2 studies and five parallel Phase 3 studies of either indiplon-IR or indiplon-MR were used in a dose–response assessment of the effect of indiplon on sleep maintenance. The data included 2,492 subjects and dosages ranging from 5 to 40 mg. It was concluded that a dose range of 5–15 mg was optimal for the subjective total sleep time outcome in insomnia subjects, and that for older subjects lower doses may be required [26].

7 Safety and Tolerability

Assessments regarding safety and tolerability have been incorporated into multiple Phase I and II pharmacokinetic and pharmacodynamic studies. Further, the clinical efficacy trials described above have monitored potential adverse effects and, in

some studies, have monitored possible next-morning residual impairment or sedation with instruments, such as the digit symbol substitution test (DSST), symbol copy test (SCT), and visual analog scale of sleepiness (VAS). With indiplon bedtime doses, the next-morning outcomes have been similar to placebo, except in one indiplon-MR dosing study with older adults where there was a modest DSST effect present only at the highest study dose [24]. In general, the adverse effects have been similar to placebo.

A safety and tolerability study of 1 year duration was conducted with 536 chronic insomnia subjects who were randomized to double-blind, as-needed treatment with either indiplon 10 or 20 mg. The three adverse events most commonly reported for indiplon 10 mg were headache (11.8%), back pain (7.9%), and somnolence (7.9%). Among subjects taking indiplon 20 mg, the three most common adverse events were headache (8.7%), upper respiratory infection (6.5%), and nasopharyngitis (5.9%) [27].

The assessment of specific safety issues has been incorporated into various studies with both indiplon IR and MR formulations. Overall, there has been no evidence of pharmacokinetic or pharmacodynamic tolerance [7]. The 3 month indiplon-IR and indiplon-MR nightly use studies specifically did not find the presence of tolerance [18, 25]. The administration of indiplon with alcohol did not produce significant pharmacokinetic changes, and it was reported that there was little or no additive pharmacodynamic effect [10]. The pharmacokinetic profiles of the IR and MR formulations minimize the risk for additive effects with coadministered medications, other than a predictable cumulative effect with other CNS depressants.

The possibility of respiratory effects associated with indiplon-MR was assessed in healthy subjects and COPD patients exposed to a CO₂ challenge. During the daytime, 12 healthy individuals were given doses of indiplon-MR 30 mg or codeine 60 mg, which served as an active control [28]. In contrast to the codeine administration, with indiplon, there were no statistically or clinically significant effects on respiratory function. In a study with 18 mild-to-moderate COPD patients, indiplon-MR 20 mg and placebo were compared [28]. No significant oxygen saturation or respiratory disturbance index changes were found.

Safety issues associated with the middle-of-the-night dosing of indiplon-IR were assessed in a five-way crossover study where healthy adult subjects ($N = 35$) without a history of insomnia were given either indiplon-IR 10 mg, indiplon-IR 20 mg, zolpidem 10 mg, zopiclone 7.5 mg, or placebo during an awakening 4 h following their bedtime. Assessments of potential next-day residual effects included the DSST, SCT, and VAS 4 and 6 h postdose. No significant differences between the indiplon doses and placebo were evident [29]. A similar study was performed with healthy older adults (ages 65–80 years) given postbedtime doses of indiplon-IR 5 and 10 mg, placebo, and zopiclone 3.75 mg. Potential impairment reflected by a DSST score reduction was evident only with the 10 mg dose and only at the 4 h postdose assessment. This was in contrast with the zopiclone DSST reduction measured at 4 and 8 h postdose. The investigators concluded that

indiplon-IR doses of 10 mg in adults and 5 mg in older adults were not associated with next-day residual sedation or impairment when given 4 h prior to awakening [30].

Psychomotor, subjective, and cognitive parameters were assessed in a study of relative abuse liability comparing indiplon (30–80 mg) and triazolam (0.25–0.75 mg). The subjects were volunteers with a history of sedative abuse. It was concluded that indiplon and triazolam shared similar abuse potential, but that the possible psychomotor and cognitive impairment following large doses might be of shorter duration with indiplon [31].

8 Comments

The constellation of pharmacokinetic, pharmacodynamic, safety, and efficacy data suggest that indiplon should be an effective hypnotic that is well tolerated. The two indiplon formulations – immediate-release and modified-release – in clinical trials consistently demonstrated significant objective and subjective efficacy benefits with parameters representing sleep onset, sleep maintenance, and sleep quality. The sleep-promoting efficacy at nighttime was achieved without causing next-morning residual sleepiness or impairment. The clinical trials evaluating indiplon have included both acute and chronic treatment in both adults and elderly subjects. The indiplon Phase I, II, and III trial results generally support a positive safety profile for both the immediate and modified-release formulations. The studies suggest that there is minimal potential for adverse effects, tolerance, respiratory depression, and drug–drug interactions.

The available evidence suggests that indiplon-IR should promote a rapid sleep onset but have minimal effects within a few hours. A medication with this profile should be especially useful for people primarily experiencing difficulty falling asleep or for those suffering with sleep maintenance insomnia and desiring a short-acting middle-of-the-night hypnotic dose. A hypnotic with the combination of rapid onset of action and short duration also should be beneficial for people whose schedules allow limited opportunities for sleep, as may occur with some shift workers. Insomnia patients with the common combined problems of difficulty initiating and maintaining sleep should achieve greater benefit from the modified-release indiplon formulation.

Indiplon shares multiple characteristics with other hypnotic medications in being an $\alpha 1$ -selective allosteric modulator of GABA_A responses. Among available medications, the indiplon-IR formulation is most similar to zaleplon; however, indiplon may act with greater potency in promoting sleep onset due to its high affinity for the receptor. Another unique potential advantage for indiplon is that middle-of-night dosing with the immediate-release formulation has been studied for efficacy and safety, and potentially this use could represent a specific clinical indication. If approved by the FDA, it is likely that there will be no implied limitation on the duration of use, as this has been the case for all insomnia treatment medications

approved by the FDA since 2005. The immediate-release and modified-release indiplon preparations have unique characteristics that should make them welcome additions to the currently available medications indicated for the treatment of insomnia.

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Polysomnographic and Clinical Assessment of Zaleplon for the Treatment of Primary Insomnia

Joseph Barbera and Colin Shapiro

Abstract Insomnia is a heterogeneous phenomenon, defined as difficulty in initiating and maintaining sleep, and nonrestorative sleep. Chronic insomnia is a highly prevalent condition with a high level of psychosocial, occupational, health, and economic morbidity. The treatment of insomnia includes both nonpharmacologic and pharmacologic measures.

The pharmacologic treatment of insomnia in the last two decades has been revolutionized by the development of the nonbenzodiazepine hypnotics, also known as the Z-drugs, including zopiclone, eszopiclone, zolpidem, and zaleplon. These drugs have proven to offer a more favorable benefit to risk profile than the benzodiazepines that preceded them, owing to their selectivity for the BZ_1/ω_1 receptor subtype of the $GABA_A$ receptor complex.

Zaleplon is a hypnotic with a unique pharmacologic profile owing to an ultra-short half life and its selective binding of the BZ_1/ω_1 receptor subtype of the $GABA_A$ receptor complex. A number of studies have demonstrated the polysomnographic and clinical features of zaleplon, many predicted by its unique pharmacologic profile. Zaleplon appears to be effective in the initiation of sleep onset, with less effect on sleep maintenance parameters. It is associated with minimal next day sedative, psychomotor and memory effects, with both evening and middle-of-night dosing. It appears to possess a relatively low potential for tolerance, withdrawal, and rebound effects; abuse potential, respiratory depression, other adverse effects, and sleep architecture changes. Zaleplon, with its unique pharmacologic profile of zaleplon, offers another option to clinicians in the treatment of insomnia.

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1 Introduction

Insomnia is a heterogeneous phenomenon defined as difficulty in initiating and maintaining sleep (with multiple awakenings during the night or premature termination of sleep) or chronic, nonrestorative sleep, with most patients with insomnia suffering from a combination of these disturbances. Nocturnal symptoms are in turn accompanied by a number of residual daytime symptoms including fatigue, decreased energy, daytime sleepiness, poor concentration, memory impairment, reduced ability in performing complex tasks, and mood disturbances such as irritability and depression [1]. Chronic insomnia is a highly prevalent condition, with a high level of psychosocial, occupational, health, and economic morbidity [2].

The treatment of chronic insomnia includes nonpharmacologic strategies falling under the category “cognitive behavioral therapy” and pharmacologic agents [3]. The mainstay of pharmacologic treatment of insomnia in the 30 years prior to the last two decades was the benzodiazepines, which possessed an overall superior benefit to risk profile in comparison with the barbiturates and chloral hydrate that preceded them. The actions of the benzodiazepines are mediated through nonselective activation of the BZ_1/ω_1 and BZ_2/ω_2 receptor subtypes of the $GABA_A$ receptor complex, providing them with hypnosedative, anxiolytic, anticonvulsant, and myorelaxant properties. While benzodiazepines have been shown to be effective in increasing sleep duration [4], they have also been associated with a number of untoward side effects including tolerance, dependence, withdrawal, and abuse potential; impairment in daytime cognitive and psychomotor performance (including an increased risk of accidents and falls); adverse effects on respiration; and disruption of normal sleep architecture with a reduction in both slow wave sleep and REM sleep [5, 6]. In the last decade, the benzodiazepines have largely been supplanted by a series of nonbenzodiazepines, benzodiazepine receptor agonists that have demonstrated a greater selectivity for the BZ_1/ω_1 receptor subtype, thus retaining the hypnosedative properties of the benzodiazepines while avoiding some of their side effects and alternative effects. This next generation of hypnotics, also known as the Z-drugs, include the hypnotics zopiclone, eszopiclone, zolpidem, and zaleplon [5–7].

Despite the advances of these newer hypnotic agents, a key problem in the treatment of insomnia continues to be its phenomenological heterogeneity. Patients presenting with predominantly difficulties initiating sleep may be treated with agents that are effective in initiating sleep, but owing to their long half-life, lead to residual daytime symptoms. Particularly problematic are cases of insomnia characterized predominantly by difficulties maintaining sleep. In such cases, clinicians are left with the task of finding an agent that is sufficiently effective over the entire course of the night, in order to mitigate nocturnal awakenings or early morning awakenings, while at the same time free of residual daytime effects.

Zaleplon is a pharmacologic agent with a unique pharmacologic profile, possessing an ultrashort half life and selective binding of the BZ_1/ω_1 receptor subtype of the $GABA_A$ receptor complex. This paper reviews the pharmacology of this unique

pharmacologic agent and its polysomnographic and clinical consequences. We have published previous reviews of zaleplon elsewhere [8, 9].

2 Pharmacologic Profile

Zaleplon is a nonbenzodiazepine, benzodiazepine receptor agonist, belonging to the pyrazolopyrimidine drug class (see Fig. 1), which also includes the hypnotic indiplon. As demonstrated by a number of nonclinical studies, zaleplon binds selectively to the BZ_1/ω_1 receptor subtype on the $GABA_A$ receptor complex (which has also been equated to $GABA_A$ receptors containing the α_1 subunit) [7, 10–13].

In humans, zaleplon is rapidly absorbed with a peak onset (t_{max}) of approximately 1 h after oral administration [14–17]. The drug undergoes significant first pass metabolism, with an absolute bioavailability of 30% [16]. The metabolism of zaleplon occurs in the liver, primarily by aldehyde oxidase into 5-oxo-zaleplon, and secondarily by CYP3A4 into desethylzaleplon, which is further metabolized by aldehyde oxidase into 5-oxodesethylzaleplon. All three metabolites are considered pharmacologically inactive. Less than 1% of zaleplon is excreted unchanged into the urine [16, 18]. The most striking pharmacokinetic feature of zaleplon is its rapid elimination, with an elimination half life ($t_{1/2}$) of 1 h [14–17]. By comparison, the elimination half life of the short-acting benzodiazepine triazolam is 2.5 h and that of zolpidem 2–2.2 h and zopiclone 4–5 h [6, 19]. The elimination half-life of zaleplon does not seem to be affected by age [20]. The clearance of zaleplon is significantly reduced in patients with liver disease [21] but not in those with renal impairment [22].

3 Clinical Efficacy

The therapeutic efficacy of zaleplon has been investigated in a number of studies with both evening and middle-of-the-night-dosing, during daytime naps, and for acute sedation in the elderly and in special populations. Outcome measures in such

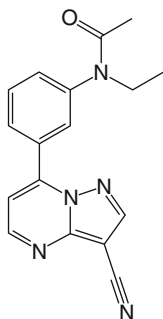


Fig. 1 Zaleplon

studies have employed objective measures in the form of polysomnography as well as subjective ratings of improved sleep.

3.1 Evening Dosing

A number of randomized, placebo-controlled trials have examined the efficacy of zaleplon in improving sleep parameters in patients with primary insomnia with evening dosing.

Walsh et al. [23] examined the efficacy of zaleplon 5 mg, zaleplon 10 mg in comparison to placebo, with triazolam 0.25 mg as an active comparator in a group of 132 adult patients with regular evening dosing over a period of 14 days. Outcome measures included polysomnographic recording (PSG) on the first and last 2 nights of treatment as well as subjective measures. Zaleplon 5 and 10 mg both significantly shortened the latency to persistent sleep in comparison with placebo on polysomnography during the first 2 night of treatment. The effect of zaleplon on sleep latency remained consistent during the last two PSG recorded nights, but such effects were no longer significant in comparison to placebo. Total sleep time and the number of awakenings were not affected by either zaleplon dose. Subjective measures supported the objective findings but were only statistically significant for zaleplon 10 mg. Triazolam 0.25 mg in comparison to placebo had similar effects to zaleplon on latency to persistent sleep with an additional improvement in total sleep time at the beginning of the study.

Elie et al. [24] studied the effects of zaleplon 5, 10, and 20 mg in comparison to placebo in a group of 574 patients with primary insomnia over 4 weeks, with zolpidem 10 mg as an active comparator. Outcome sleep variables were measured subjectively using postsleep questionnaires. Median sleep latency was significantly reduced for zaleplon 5 mg in comparison to placebo over the first 3 weeks of treatment, and for zaleplon 10 and 20 mg over all 4 weeks. Only zaleplon 20 mg had an effect on total sleep time in all weeks, except in week 3. By comparison, zolpidem 10 mg decreased sleep latency during the first 3 weeks of treatment, and increased total sleep time over all 4 weeks. Mixed results were seen in all treatment groups with respect to subjective quality of sleep, and no treatment affected the number of awakenings during the night.

In a similar protocol, Fry et al. [25] investigated the effects of zaleplon 5, 10, and 20 mg in comparison to placebo in 586 patients with primary insomnia over 4 weeks of treatment, with zolpidem 10 mg as an active comparator. Subjective sleep latency was significantly reduced in comparison to placebo for zaleplon 5 mg only at week 1, for zaleplon 10 mg at weeks 1 and 3, and for zaleplon 20 mg over all 4 weeks. Only zaleplon 20 mg produced significant, but variable, effects on subjective total sleep time, sleep quality, and number of awakenings during the night. Zolpidem 10 mg reduced sleep latency only on weeks 1 and 4, with variable effects on total sleep time and sleep quality.

26In a 2-night treatment protocol, Drake et al. [26] examined the effects of zaleplon 10, 20, 40, and 60 mg and triazolam 0.25 mg in comparison to placebo in 83 patients with primary insomnia, with polysomnography being conducted on both treatment nights. All zaleplon doses as well as triazolam 0.25 mg were found to significantly reduce latency to persistent sleep on polysomnography recording in comparison to placebo (with triazolam 0.25 mg having comparable effects to zaleplon 10 mg). Only zaleplon 60 mg and triazolam 0.25 increased total sleep time in comparison to placebo. A number of subjective measures supported the objective PSG findings.

In a 5-week treatment protocol, Walsh et al. [27] studied the effects of zaleplon 10 mg in comparison with placebo in 113 patients with primary insomnia. Polysomnography was employed at baseline and on the first 2 nights of each treatment week, in addition to subjective measures. Zaleplon 10 mg significantly reduced the PSG-derived latency to persistent sleep during all 5 weeks in comparison to placebo, as assessed by PSG and subjective measures. No consistent treatment effects were noted in terms of total sleep time, number of awakenings, or wake time after sleep onset.

3.2 *Middle-of-the-Night Dosing*

The ultrashort half life of zaleplon has led to its being advocated for middle-of-the-night use, as needed, in patients with sleep maintenance insomnia. Few studies, however, have specifically examined the use of zaleplon in this regard. 28In a single night protocol with 28 healthy volunteers, Vermeeren et al. [28] reported that zaleplon 10 and 20 mg administered 5 h after sleep onset increased subjective ease of initiating sleep and sleep quality in the second half of the night. Walsh et al. [29] reported that zaleplon 10 mg administered 3.5 h after bedtime significantly reduced sleep latency but had no effect on total sleep time in 22 patients with sleep maintenance insomnia, during two PSG-recorded nights. Stone et al. [30] reported that zaleplon 10 and 20 mg significantly reduced latency to persistent sleep when administered after 5 h of sleep during a single PSG-recorded night in an experimental, noise-induced model of situational insomnia with 13 normal subjects. Hindmarch et al. [31] reported that in 40 normal subjects, zaleplon 10 and 20 mg significantly improved subjective ease of getting to sleep when given 1 h before awakening, and mixed results on perceived sleep quality when given 3 and 5 h before awakening. Zammit et al. [32] reported that zaleplon 10 mg given 4 h after bedtime resulted in significant reduction in latency to persistent sleep, and increased total sleep time, in 37 adults with sleep maintenance insomnia during two PSG-recorded nights. It is notable that all of the preceding studies employed forced awakenings to assess middle-of-the-night use of zaleplon, which may not exactly correlate to naturally occurring middle-of-the-night awakenings [32].

3.3 Daytime Naps/Acute Sedation

The short half life of zaleplon would also make it suitable for daytime naps where rapid recovery is required, such as in military or emergency personnel, or for acute sedation during medical procedures. Only a limited number of studies, however, have directly examined the effects of zaleplon in this regard.

Whitmore et al. [33] investigated the effects of zaleplon 10 mg versus placebo on a 3.5 h afternoon nap opportunity in 12 healthy subjects, employing a modified PSG recording. Zaleplon 10 mg significantly increased slow wave sleep during naps with a marginal, but nonsignificant, increase in total sleep time. Simons et al. [34] compared zaleplon 10 mg, temazepam 20 mg, and placebo on a 4.5 h afternoon nap opportunity in 11 healthy subjects, employing actigraphy and subjective measures. Zaleplon 10 mg did not produce any significant effects on measures of sleep quality and total sleep time, in contrast to temazepam 20 mg.

Only one study has examined the effects of zaleplon sedation for medical procedures. Ganzberg et al. [35] compared zaleplon 10 mg to triazolam 0.5 mg with respect to sedation during a 3rd molar extraction in 14 otherwise healthy subjects. zaleplon 10 mg was found to be comparable to triazolam 0.5 mg on subjective measures of anxiolysis and sedation (patients, notably, did not actually fall asleep during the procedure).

3.4 Elderly Patients

A number of randomized placebo-controlled trials have investigated the efficacy of zaleplon in elderly patients with evening dosing. Walsh et al. [36] administered zaleplon 2, 5, and 10 mg in a group of 48 elderly patients with insomnia during two PSG-recorded nights. The latency to persistent sleep was significantly reduced in with all zaleplon doses in comparison to placebo on PSG recording and in the zaleplon 5 and 10 mg groups with subjective estimates. Total sleep time was significantly reduced in comparison to placebo with zaleplon 5 and 10 mg on PSG recording and with zaleplon 10 mg with subjective estimates. No treatment effects were seen on the number of awakenings during the night or in subjective sleep quality.

Hedner et al. [37] studied the effects of zaleplon 5 and 10 mg on 422 elderly patients with chronic primary insomnia over a period of 2 weeks using postsleep questionnaires. Subjective sleep latency was significantly reduced for both 5 and 10 mg in comparison to placebo during both treatment weeks. Subjective total sleep time was significantly increased for zaleplon 10 mg during the first week. A small but significant effect on subjective number of awakenings during the night was also seen with zaleplon 10 mg during the first week. Small but significant improvements in subjective sleep quality were seen with zaleplon 5 and 10 mg during both treatment weeks.

In a similar study, Ancoli-Israel et al. [38] investigated the effects of zaleplon 5 and 10 mg in comparison to placebo on 549 elderly patients with primary insomnia over 2 weeks of treatment, with zolpidem 5 mg as an active comparator. Zaleplon 10 mg significantly reduced subjective sleep onset latency on both treatment weeks, while zaleplon 5 mg only did so on week 2. Subjective total sleep time was significantly increased with zaleplon 10 mg during week 1. Zolpidem 5 mg produced significant effects on subjective sleep latency, total sleep time, and number of awakenings during both treatment weeks.

3.5 Special Populations

The majority of studies investigating the efficacy of zaleplon have been conducted on otherwise healthy patients with primary insomnia or on healthy normals. A limited number of studies have been conducted with zaleplon on special populations. Schwartz et al. [39] found zaleplon 10–20 mg to be significantly less effective than trazodone 50–100 mg in increasing the quality of sleep in 15 psychiatric inpatients, although zaleplon was associated with less residual daytime sedation. Sabbatinin et al. [40] investigated the effects of zaleplon 10 mg versus placebo in 14 hemodialysis patients in 2 week crossover design. Zaleplon 10 mg in comparison to placebo significantly improved subjective sleep quality, sleep latency, and habitual sleep efficiency, but not duration of sleep. Beaumont et al. [41] investigated the effects of zaleplon 10 mg, zolpidem 10 mg, and placebo on 12 healthy males at high altitude (3,613 m) in a 3-night PSG-recorded crossover protocol. Zaleplon 10 mg, as with zolpidem, resulted in significant improvements in wakefulness after sleep onset and sleep efficiency.

In summary, a number of studies have demonstrated the efficacy of zaleplon in reducing sleep latency with evening dosing in both elderly and nonelderly patients. While the use of zaleplon is frequently advocated for middle-of-the-night use, only a limited number of studies support its use in this regard. Consistent with its short half life, zaleplon when given at standard recommended doses (10 mg in nonelderly patients, 5 mg in elderly patients) has little effect on total sleep time or other sleep maintenance parameters.

4 Adverse Effects

A number of studies have examined the safety of zaleplon, particularly with respect to residual daytime effects, in comparison with other, longer-acting, standard hypnotics. Such studies have focused on the effects of zaleplon on psychomotor and memory impairment, driving impairment, tolerance, rebound and withdrawal, and other adverse effects including abuse potential, drug interactions, and respiratory depression.

4.1 Sedation, Psychomotor/Memory Impairment

A significant number of studies have investigated the effects of zaleplon on sedation (subjective and objective) as well as psychomotor/memory functioning, employing a number of standard tests (e.g., digit symbol substitution test, critical flicker fusion, psychomotor vigilance test, word list recall, etc.).

Several studies have investigated the immediate short-term effects of zaleplon following daytime dosing in awake, healthy subjects. Such studies have employed doses of zaleplon in doses of 1–60 mg and have demonstrated little sedation, psychomotor/memory impairment beyond the 2–4 h range [14, 15, 17, 42–44]. Moreover, zaleplon was associated with less impairment and more repaid recovery from impairment compared to lorazepam 2 mg [42], zolpidem 10–20 mg [15, 17], zopiclone 7.5 mg [43, 44] and temazepam [43].

Not surprisingly, evening dosing of zaleplon (5, 10 and 20 mg) has also been shown to have relatively little, if any, effect on morning sedation and psychomotor/memory impairment, at least based on a number of short-term studies (1 night to 2 weeks) on normal subjects or healthy insomniacs [23, 26, 28, 45, 46], including elderly patients in doses up to 10 mg [36]. Zaleplon in this regard was demonstrated to be more favorable to therapeutic doses of zolpidem [45], triazolam [26, 45], and zopiclone [28, 46].

In similar short-term studies, middle-of-the-night use of zaleplon has also been shown to cause relatively little morning sedation and psychomotor/memory impairment with dosing up to hours before morning testing [28–32, 47, 48]. Zaleplon in this regard was significantly more favorable than therapeutic doses of zopiclone [28, 30], flurazepam [29], and zolpidem [31, 32, 47, 48]. Similarly, zaleplon has been shown to have relatively short lived or absent residual effects following induced afternoon napping [33, 34, 49] or acute sedation [35].

4.2 Driving Impairment

Vermeeren et al. [28] investigated the effects of zaleplon in comparison with zopiclone on an actual driving task in 28 healthy volunteers. No driving impairment was noted for both evening and middle-of-the-night use of zaleplon 10 and 20 mg, in contrast to zopiclone 7.5 mg, which results in significant driving impairment, particularly with middle-of-the-night dosing. Using data from this study, and an analytic model of equivalent blood alcohol concentration (BAC), Menzin et al. [50] estimated that zopiclone, in comparison to zaleplon, would be expected to result in 503 excess accidents per 100,000 drivers over 14 days in France. In a similar study, Vermeeren et al. [46] found that evening dosing of zaleplon 10 mg was not associated with significant driving impairment, in contrast to evening zopiclone 7.5 mg, which was judged to be worse than the effects of low dose alcohol (BAC 0.05%). Verster et al. [47] in another driving study found that middle-of-the-night

dosing of zaleplon 10 and 20 mg was not associated with significant driving impairment 4 h after dosing. This was in contrast to middle-of-the-night dosing of zolpidem 10 and 20 mg, which was judged to be worse than the effects of low dose alcohol. A meta-analysis of these studies found a lack of morning driving impairment for zaleplon 10 and 20 mg with both evening and middle-of-the-night administration [51].

One study has reported a case of a motor vehicle accident associated with the misuse of 60 mg of zaleplon [52].

4.3 Tolerance, Rebound, and Withdrawal

In efficacy studies already noted, zaleplon 5–20 mg was found to be relatively persistent in terms of efficacy over a period of 2–5 weeks, suggesting a lack of tolerance over that period [23–25, 27]. An absence of rebound or withdrawal has been noted for zaleplon 5–20 mg during 2–3 discontinuation nights [23–25, 27] in nonelderly adults. Scharf [53] reported that in two open label follow up studies, the effectiveness of zaleplon 10 mg was maintained over 12 months of use, with no evidence of withdrawal on discontinuation.

In studies on elderly patients, weak rebound effects were noted for zaleplon 10 mg but not 5 mg on the first discontinuation night [37, 38]. In open-label studies in elderly patients, the efficacy of zaleplon 5–10 mg has been reported to remain persistent over 6–12 months, with little evidence of rebound insomnia on discontinuation [54, 55].

Given the short half-life of zaleplon, withdrawal effects within a single night would be a theoretical possibility. However, Walsh et al. [27] did not find an effect of evening zaleplon 10 mg on minutes of wake time in the last quarter of an 8 h night. Vermeeren et al. [28] reported that evening dosing of zaleplon had no effect on subjective measures in the second half of the night, in comparison with placebo.

4.4 Abuse Potential

While a lack of tolerance and rebound suggests a lack of physical dependence with respect to the use of zaleplon, few studies have investigated its abuse potential in humans. Beer et al. [14] found no difference between the use of zaleplon at doses up to 60 mg and placebo on a “drug liking” questionnaire. Rush et al. [56] investigated the abuse potential of zaleplon on a group of 14 subjects with histories of drug abuse (including sedatives and hypnotics) using a series of drug effect questionnaires. The authors found comparable dose-related effect with respect to abuse potential and behavioral pharmacological profiles for both drugs, but employed relatively high doses of zaleplon (25, 50, and 75 mg) in comparison to relatively

modest doses of triazolam (0.25, 0.50 and 0.75 mg). The abuse of high doses of zaleplon, taken intranasally and orally, has been reported in case reports [52, 57].

4.5 Drug Interactions

Potentially relevant pharmacodynamic and pharmacokinetic interaction with zaleplon would be expected with other CNS active agents (including sedatives and other psychotropics), medications with common metabolic pathways with that of zaleplon (CYP inducers and inhibitors), medications with low therapeutic tolerances (digoxin and warfarin), and those that effect renal excretion (ibuprofen) [58].

Zaleplon at doses of 10–20 mg appears to be relatively safe in combination with medications studies to date, although a paucity of such studies does exist for zaleplon, even in comparison with other nonbenzodiazepines zolpidem and zopiclone. In such studies, no pharmacokinetic interactions between zaleplon and alcohol, imipramine and thioridizine, were detected, while the additive pharmacodynamic effects with these drugs usually resolved within 2–4 h. No significant pharmacodynamic or pharmacokinetic effects were found between zaleplon and paroxetine. Diphenhydramine (50 mg), a moderate inhibitor of aldehyde oxidase, was not found to affect the pharmacokinetics of zaleplon. Cimetidine (800 mg), a moderate inhibitor of both aldehyde oxidase and CYP3A4, decreased the oral-dose clearance (CL/F) of zaleplon by 44%, and increased its C_{\max} and AUC by 85%. Rifampin, a CYP inducer, increased the CL/F of zaleplon by 5.4-fold, with a decrease in the C_{\max} and AUC of zaleplon by 80%. Zaleplon has been shown to increase the C_{\max} of (S–)-warfarin by 17%, but had no effect on the pharmacokinetics of (R+)-warfarin. Warfarin-induced prolongation of the INR was not altered by coadministration of zaleplon. No pharmacokinetic interaction was noted between zaleplon and ibuprofen [58, 59].

Cases have been reported of medication-induced comas with zaleplon in combination with alcohol and trimipramine [60]. A case has also been reported of a suicide by drug intoxication involving high doses of zaleplon, butalbital, and alprazolam [61].

4.6 Respiratory Depression

Caution is recommended in using benzodiazepines in the treatment of insomnia in patients with respiratory disorders such as chronic obstructive pulmonary disease (COPD) and obstructive sleep apnea (OSA), owing to their potential to decrease respiratory effect, decrease upper airway muscle tone, and blunt arousal response to hypoxia/hypercapnia [62, 63]. A limited number of studies have examined this issue with respect to zaleplon. George [64] did not find any significant effects of zaleplon 10 mg on oxygen saturation indices and apnea/hypopnea index over three

PSG-recorded night in 31 insomniacs with mild to moderate COPD. George [62] found no significant difference between zaleplon 10 mg and placebo in nine outpatients with mild to moderate obstructive sleep apnea with respect to the apnea/hypopnea index and oxygen saturations indices over two PSG-recorded nights. Coyle et al. [65] found no significant difference between zaleplon 10 mg and placebo in 15 mild to moderate obstructive sleep apnea patients for the apnea/hypopnea index and mean S_pO_2 over 5 nights of recording. While the authors did find a treatment effect for the nadir S_pO_2 , this was judged to be of unlikely clinical significance.

In a study on 12 healthy males in a simulated altitude environment (4,000 m), zaleplon 10 mg was not found to have any significant effect on PSG-recorded respiratory parameters [66]. In a study on 12 healthy males at an actual high altitude environment (3,613 m), zaleplon 10 mg was also shown to have no effect on respiratory parameters [41].

4.7 Other Adverse Effects

A number of neurological-related adverse effects have been reported with the use of zaleplon including drowsiness/somnolence [14, 27], dizziness [14, 25], and visual hallucinations [42, 67]. The most commonly reported adverse event with zaleplon was headache, although the incidence of this was generally no more significant than that of placebo [23–25, 27]. In no study were significant effects noted with zaleplon on vital signs, ECG, hematologic, and clinical chemistry parameters. One report has described a case of somnambulism and complex behavior following an overdose of zaleplon [68].

5 Effects on Sleep Architecture

A number of studies have examined the effects of zaleplon on sleep architecture, as measured by polysomnography.

Walsh et al. [23] noted that the percentage of total sleep time spent in each stage of sleep for the entire night, and for quarters of the night, was unaltered with both evening dosing of both zaleplon 5 and 10 mg, both at the beginning of treatment and at the end of 2 weeks of treatment. A similar absence of sleep architecture effects was noted with triazolam 0.25 mg. Walsh et al. [27] did not find any significant differences between evening zaleplon 10 mg and placebo on the percentages of stage 1, stage 2, or stage 3/4 sleep over 5 weeks of treatment. During week 2 of treatment, the percentage REM sleep was found to be significantly lower with zaleplon 10 mg, and during week 4 of treatment, REM sleep latency was significantly longer with zaleplon 10 mg in comparison to placebo.

Stone et al. [30] during a single night of treatment noted that middle-of-the-night dosing of zaleplon 20 mg reduced the duration of stage 1 sleep in comparison with placebo. Zopiclone 7.5 mg, in contrast, reduced the duration of stage 1 sleep, and increased the duration of stage 3 sleep. No significant effects were noted on the percentages of all stages of sleep for zaleplon 10 and 20 mg, and zopiclone 7.5 mg.

Drake et al. [26], during 2 nights of treatment, found significant increases in the percentage of stage 3/4 sleep with evening zaleplon 40 and 60 mg in comparison to placebo and triazolam 0.25 mg. Zaleplon 40 mg and 60 mg also reduced the percentage of REM sleep in comparison with placebo. Triazolam 0.25 mg, by contrast, reduced both the percentages of stage 3/4 and REM sleep in comparison with placebo. Sleep architecture with zaleplon 10 and 20 mg was well preserved in comparison to placebo.

In elderly patients, Walsh et al. [36] did not find any effects of zaleplon 2, 5, and 10 mg on the total sleep time or percentages of stages 1 and 2 slow wave sleep or REM sleep during 2 nights of treatment. Significant treatment effects were noted on REM sleep latency, such effects occurring in a dose-dependent manner with increasing doses producing greater latency. Zaleplon 5 mg significantly increased REM latency in comparison to placebo.

Whitmore et al. [33] found that the use of zaleplon 10 mg to induce an afternoon nap in well rested individuals resulted in a significant increase in total slow-wave sleep (stage 3 and 4) in comparison to placebo, despite only a marginal, nonsignificant increase in total sleep time.

Beaumont et al. [41], in a high altitude (3,613 m) treatment protocol, found that zaleplon 10 mg was associated with an increase in the duration of slow wave sleep in the first half of the night, in comparison with placebo. Zolpidem 10 mg, by contrast, was associated with a significant increase in the duration of slow wave sleep throughout the night. In a previous study, a simulated high altitude (4,000 m) protocol did not find a significant effect of zaleplon 10 mg on SWS, in contrast to zolpidem 10 mg, although a significant effect on stage 4 sleep was noted for zaleplon [66]. In both studies, high altitude was associated with a reduction in slow wave sleep in comparison to sea level, and any treatment effects were actually a mitigation of the reduction in slow wave sleep occurring at high altitudes [41, 66].

In summary, zaleplon appears to be associated with a relative preservation of sleep architecture at doses of 5–20 mg in nonelderly adults. Higher doses may increase slow wave sleep and decrease the percentage of REM sleep. Standard doses of zaleplon may also produce short-term changes in slow wave sleep (such as during naps or in the first half of the night).

6 Conclusion

Zaleplon is a hypnotic with a unique pharmacologic profile, owing to its ultrashort half life and selective binding of the BZ_1/ω_1 receptor subtype of the $GABA_A$ receptor complex. As predicted by this unique profile, zaleplon has been shown

in a number of studies to exhibit a number of polysomnographic and clinical features. In keeping with its short half life, zaleplon appears to be efficacious in promoting sleep onset, while less effective in maintaining sleep. Also in keeping with its short half life is a minimum of residual daytime sedative, psychomotor, and sedative effects, with both evening and middle-of-the-night dosing. In keeping with its BZ_1/ω_1 receptor selectivity, zaleplon seems to possess a relatively low potential for tolerance, withdrawal, and rebound effects; abuse potential, respiratory depression, other adverse effects, and sleep architecture changes.

It has been said that the ideal hypnotic should have a rapid onset of action to promote sleep initiation, have a sustained effect to promote maintenance, and be free of residual next-day effects. Unfortunately, no such hypnotic exists and clinicians are left with finding a balance between maximizing sleep duration over the course of a night while minimizing next day residual side effects. Zaleplon, with its unique pharmacological profile provides one more tool in the clinician's armamentarium. The short half life of zaleplon makes it suitable for patients with predominantly difficulties initiating sleep. This short half life also makes it uniquely suitable for "middle-of-the-night administration" in patients with isolated nocturnal awakenings or premature termination of sleep, who require a medication in order to reestablish sleep, without the significant effects of residual daytime effects associated with longer acting agents. Zaleplon may also be of benefit to patients exposed to short sleep opportunities or unpredictable awakenings requiring maximal alertness (e.g., military personnel, firemen etc.). Zaleplon thus offers another option in the management of patients with insomnia.

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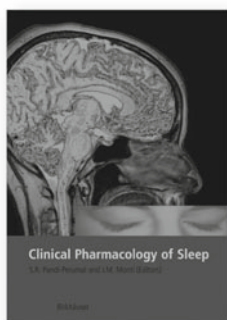
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Clinical Pharmacology of Sleep

Pandi-Perumal, S.R., Comprehensive Center for Sleep Medicine, New York, USA / **Monti, J.M.**, Pharmacology and Therapeutics, Montevideo, Uruguay (Eds)

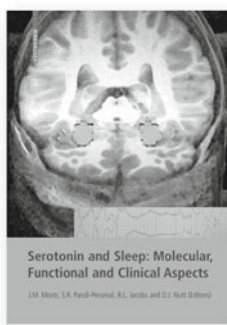
Pandi-Perumal / Monti (Eds)
Clinical Pharmacology of Sleep
2006, XII, 239 p. 6 illus.,
Hardcover
ISBN 978-3-7643-7262-0

The field of sleep medicine is growing rapidly, and the clinical pharmacology of sleep is gaining much attention from sleep physicians. Many new drugs are in the process of being developed and tested for their possible usefulness in the treatment of sleep disorders. The opportunity for further advancement in this field is very promising.

This volume covers the clinical and pharmacological treatment of several important sleep disorders such as insomnia, sleep apnea, narcolepsy, restless legs syndrome, and periodic limb movement syndrome. It further addresses the use of sleep medications in children, adolescents, and in the elderly. It offers a comprehensive overview of the currently available hypnotic medications and covers aspects of chronopharmacology and its implications for the pharmacology of sleep. It also reviews the basic science of sleep and sleep disorders, and thus the potential development of new pharmacological approaches.

From the contents:

Preface.- Primary and secondary insomnia.- Primary insomnia: diagnosis and treatment.- Neuropharmacology of obstructive sleep apnea and central apnea.- Narcolepsy syndrome: a new view at the beginning of the second millennium.- Sleep disturbances in restless legs syndrome (RLS) and periodic limb movements (PLM).- Sleep disturbances in anxiety disorders.- Sleep disturbances in affective disorders.- Sleep disturbance in schizophrenia.- Clinical pharmacology of sleep disturbances in children and adolescents.- Assessment and treatment of sleep disturbances in aged population.- Sleep disturbances in Alzheimer's disease.- Sleep disturbance during menopause.- Chronopharmacology and its implications to the pharmacology of sleep.- Overview of currently available benzodiazepine and nonbenzodiazepine hypnotics.- Rebound and withdrawal with benzodiazepine and non-benzodiazepine hypnotic medications.- Index.



Serotonin and Sleep: Molecular, Functional and Clinical Aspects

Monti, J.M.; Pandi-Perumal, S.R.;
Jacobs, B.L.; Nutt, D.J. (Eds.)

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2008, XXVIII, 621 p. 80 illus.,
3 in color., Hardcover
ISBN 978-3-7643-8560-6

Serotonin and Sleep: Molecular, Functional, and Clinical Aspects covers the complete spectrum of the field and explores the link between the latest basic molecular, functional, and clinical aspects of serotonin and the practice of sleep medicine. The volume focuses on 24 different areas of research, with special emphasis relating the state of basic and clinical research to potential applications: changing concepts in serotonin research, topographic organization and chemoarchitecture, receptor mechanisms – its organization and regulation of behavioral states, electrophysiological mechanisms – in vitro, in vivo, and behaving animals and other areas of molecular neurobiology. Also highlighted are studies related to the circadian control of behavioral states, and mechanisms involved in the serotonergic inhibition of REM sleep. Such discussion has profound implications for the basic biology of serotonin.

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Written by an international cadre of researchers, this timely volume provides an up-to-date summary of what may be the only view on serotonin from the perspectives of neurobiologists, neuroanatomists, cell biologists, psychiatrists, neuropharmacologists, as well as sleep researchers.

From the contents:

Evolution of concepts.- The dorsal raphe nucleus and median raphe nucleus: organization and projections.- Serotonin receptors.- Electrophysiology of serotonergic neurons and the regulation of serotonin release.- Serotonin receptors and the regulation of behavioural state.- Relevance of serotonin to clinical disorders and drug actions.

